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Guidelines for Authors

Aesthetic Medicine is a multidisciplinary Journal with the aim of informing readers about the most important developments in the field of Aesthetic Medicine.

Submission of manuscripts

All articles in their final version - completed with name, surname, affiliation, address, phone number and e- mail address of the author (s) - must be sent in word format to the Editorial Committee at the following e-mail address: aemj@aestheticmedicinejournal.org. Manuscripts must be written in English, and authors are urged to aim for clarity, brevity, and accuracy of information and language. All manuscripts must include a structured abstract. Authors whose first language is not English should have their manuscripts checked for grammar and stylistic accuracy by a native English speaker.

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Journal article - online* *if there is no DOI, provide the URL for the specific article	Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13- year-olds. *J Hum Nutr Diet.* 2012; 25(1): 43-49. **doi: 10.1111/j.1365-277X.2011.01184.x**
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Newspaper article - online	Pollack A. FDA approves new cystic fibrosis drug. *New York Times.* January 31, 2012. http://www.nytimes.com/2012/02/01/business/fda-approves-cystic-fibrosis-drug.html?ref=health Accessed February 1, 2012.
Websites	Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. https://www.cdc.gov Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.
Entire book - in print	Modlin J, Jenkins P. *Decision Analysis in Planning for a Polio Outbreak in the United States.* San Francisco, CA: Pediatric Academic Societies; 2004.
Book chapter - in print	Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. *Allergens and Allergen Immunotherapy.* 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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Example Article

1. Zoellner J, Krzeski E, Harden S, Cook E, Allen K, Estabrooks PA. Qualitative application of the theory of planned behavior to understand beverage consumption behaviors among adults. J Acad Nutr Diet. 2012;112(11):1774-1784. doi: 10.1016/j.jand.2012.06.368.

In-Text Citation Example

LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB,[1] it is estimated that SSB account for about 10% of total energy intake in adults[2,3]. High intake of SSB has....

References Section Example

References

1. Duffey KJ. Popkin BM. Shifts in patterns and consumptions of beverages between 1965 and 2002. *Obesity.* 2007:15(11):2739-2747.
2. Nielsen SJ. Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med.* 2004;27(3):205-210.
3. Drewnowski A. Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr.* 2007;85(3):651-661.

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Editorial

In modern years, aesthetics has become quite important in every aspect of everyday life: following the hundreds of journals, magazines, blogs and websites pointing their attention towards this interesting and fascinating topic, the request for aesthetic medicine has increased manifolds.

Aesthetic Medicine is a new field of medicine, in which different specialists share the aim of constructing and reconstructing the physical equilibrium of the individual. Treatment of physical aesthetic alterations and unaesthetic sequel of illnesses or injuries, together with the prevention of aging, are perhaps two of the most iconic areas of intervention for Aesthetic Medicine.

However, in order to prevent frailty in the elderly, a program of education is similarly important.

Furthermore, the line between health and beauty is extremely thin: psychosomatic disorders resulting from low self-esteem due to aesthetic reasons are frequent and can- not be ignored by a clinician.

It is therefore clear that there is no figure in the field of medicine which is not involved in Aesthetic Medicine: endocrinologists, gynecologists, angiologists, psychologists and psychiatrists, plastic surgeons, dermatologists, dieticians, physiotherapists, orthopedists, physical education instructors, massophysiotherapists, podologists, and rehabilitation therapists are just some of the specialists who are sooner or later going to have to answer their patients' needs for aesthetic interventions.

The involvement of all these specialists fits the description of health as defined by the WHO: "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" for which, undeniably, a team of different physicians is required.

The number of patients requiring medical consultation for esthetic reasons is rapidly increasing: in order to be able to provide adequate feedback, medical and paramedical specialists should be trained and, more importantly, should be taught how to work together. Existing Societies of Aesthetic Medicine from different countries share the aim of creating such teams and provide constant updates to the literature: the creation of an international network of specialists from all around the world under the flag of Aesthetic Medicine represents a challenge, but at the same time it is the proof of the widespread interest in this topic.

The first issue of this Journal represents the results of the efforts of the many national Societies and of the Union Internationale de Medecine Esthetique, now together as one; it is our hope that in years to come this Journal might improve our knowledge in this field, and provide adequate scientific advancement in the field of Aesthetic Medicine.

Francesco Romanelli
MD Editor-in-chief
Associate Professor at "Sapienza" University of Rome

Editors' notes

Aesthetic Medicine, the booming medical activity

Aesthetic Medicine was born in France 40 years ago.
The French Society of Aesthetic Medicine was the first of its kind in the world, followed by Italy, Belgium and Spain. Starts were rather difficult as aesthetic procedures in those early years were only surgical.
At that time aesthetic doctors and cosmetic dermatologists had very few real medical procedures to offer to their patients for treating aesthetic problems on face and body.
At the beginning of the '80s, viable medical procedures started to emerge in Europe for aesthetic and cosmetic purposes. Mostly, at that time, they were imported from the United States: those included collagen injections for wrinkles (Zyderm by Dr. Stegman), and chemical peels (phenol by Dr. Baker, TCA by Dr. Oba- gi). But, subsequently, European research on Aesthetic Medicine gained momentum. Hyaluronic acid appeared on the market, as it was discovered that it could be used as a dermal filler for wrinkles. During the '90s, the use of lasers offered aesthetic doctors and cosmetic dermatologists new possibilities.
The "beam revolution" started with CO2 laser for facial resurfacing.
Today, CO2 resurfacing is not used as much anymore, because of the long and difficult postop. CO2 laser was replaced with the gentler Nd-YAG and Erbium lasers and more recently with non invasive photonic devices for facial rejuvenation, including IPL, US and radiofrequency. These new technologies allow today's aesthetic doctors and cosmetic dermatologists to offer their patients procedures with low risk of post- op complications. Then, Botulinum Toxin has "invaded" both sides of the Atlantic Ocean.
Today, Botox injections are the most popular treatment for facial expressive wrinkles.
Botox injections are now so common everywhere that many cosmetic surgeons have given up their bistouries for syringes. Last but not least, development in Aesthetic Medicine is shown by mesotherapy and adipolipolysis.
About lipolysis, new data and recent publications have explained that radiofrequency, ultrasounds and cryolyse could have positive action to dissolve fat and to improve some unaesthetic disorders like cellulite.
These non invasive procedures intend to replace the surgical liposculpture with success.
Nowadays, Aesthetic Medicine has the necessary tools to address all major disorders within the aesthetic field. After 40 years, Aesthetic Medicine is now active in 27 countries in the world (France, Italy, Spain, Belgium, Morocco, Poland, Russia, Switzerland, Romania, Kazakhstan, Algeria, Brazil, Argentina, Uruguay, Venezuela, Colombia, Chile, Mexico, U.S.A, Canada, South Korea, and recently Ecuador, China, South Africa, Turkey, Ukraine and Georgia).
All 27 national Societies are members of the Union Internationale de M decine Esth tique (U.I.M.E.). Aesthetic Medicine is taught in 8 countries (France, Italy, Spain, Brazil, Argentina, Mexico, Venezuela, Kazakhstan) in universities that deliver UIME's diplomas after 3 to 4 years of studies.

What is the future of Aesthetic Medicine?

In the last few decades, patients' desires to look and feel younge, have fueled Aesthetic Medicine and Cosmetic Dermatology: many different procedures have been developed to satisfy the demands.
As life-span have increased, patients today are not only asking about aesthetic procedures, they are also asking for a way to stay in good physical conditions in the last decades of their lives. As a direct result, Anti-Aging Medicine, which covers skin aging and general aging, has recently emerged and expanded very quickly. Anti-Aging Medicine can offer senior patients better nutrition, dietary supplementation with vitamins, minerals, antioxidants, and eventually hormone replacement therapy, but only when needed.
Today, and in the near future, both Aesthetic Medicine and Anti-Aging Medicine will offer to our patients, who now live longer, better wellness with aesthetic treatments for skin aging and anti-aging treatments for general aging. Aesthetic Medicine is booming, but all medical practitioners should be correctly trained, so its future will be bright.

Jean-Jacques Legrand
MD General Secretary of UIME

Aesthetic Medicine: a bioethic act

When in 1977 the Italian Society of Aesthetic Medicine published the first issue of the magazine “La Medicina Estetica” Carlo Alberto Bartoletti, the Founder, wrote an editorial in which traced the pathway of the discipline and of the Scientific Society, still valid and projected into the future.
Today from that Editorial Board arise an International Journal, which wants to be indexed, in order to give to the doctors practicing Aestehetic Medicine all around the world a solid basis of shared knowledge.
In the late ‘60s, what was called in Italy Aesthetic Medicine, moved its first steps thanks to “remise en forme and anti aging projects” imported from the experience the “Institutul de geriatrie Bucuresti”, directed by Dr. Ana Aslan.
For this reason,there is the bioethical imperative that the Discipline should be first prevention, then return to physiology and finally correction.
The worldwide diffusion and the efforts of Industries born on the wave of the phenomenon have often led to choose the fastest route to achieve and maintain the physical aspect in the myth of beauty at all costs, without considering that aesthetic is not synonymous of beauty, but it is a balance between body and mind, and the role of the doctor is to take care of the Person globally and not only focusing on the correction of “a badly accepted blemish”.
Faithful to the teaching of my Master had almost 50 years ago, this new journal will have the task of elevating the human resources, aligning and validating methodologies, but above all affirming the humanitas of the medical art in its purest sense to pursue the good and the graceful for the person who relies on it.

Fulvio Tomaselli, MD
Honorary President of the Italian Society of Aesthetic Medicine

Aesthetic Medicine needs science. All over the world

All Aesthetic Doctors know that science is the basis for safety. Safety is the most important issue in our discipline.
Unfortunately, Aesthetic Medicine is more often surrounded by marketing than by science, despite the hard work done by Scientific Societies all over the World. And, too often doctors working in this field are dealing with sellers that promote products with insufficient scientific studies.
However, they sell it anyway. I think that doctors must learn that the first thing to ask about a medical device is the scientific background regarding that product: patients treated, follow up period, adverse events and, most of all, publications.
With this new International Journal completely dedicated to Aesthetic Medicine, proposed by the Italian Society of Aesthetic Medicine, endorsed by UIME and shared by all the National Societies of Aesthetic Medicine belonging to UIME, World Aesthetic Medicine wants to stimulate scientific production in this discipline to increase safety and quality in aesthetic medical procedures.
Another important goal of the Journal is to catalyze the proposal of new protocols and guidelines in Aesthetic Medicine, with the consensus of the entire Aesthetic Medicine Scientific Community.
What this Journal should achieve in the near future is to improve the number and quality of scientific production in Aesthetic Medicine, in order to allow this discipline to grow in the field of evidence based medicine, not only in the rationale field.
I hope this can be the start of a new era for Aesthetic Medicine, with the commitment of all Scientific Societies all over the world.

Emanuele Bartoletti, MD
Managing Editor
President of the Italian Society of Aesthetic Medicine

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Mini-Review

Regulation of adipose tissue function and metabolism: role of mineralocorticoid receptor

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Abstract
Mineralocorticoid receptor has been recently identified in several extra-renal tissues, including adipose tissue, where its hyperactivation contributes to some metabolic derangements frequently observed in obesity and metabolic syndrome, such as adipocyte hypertrophy, increased macrophage infiltration, oxidative stress, insulin resistance and impaired browning of white adipose tissue. Moreover, recent evidence supports the existence of a detrimental cross-talk between adipose tissue and adrenal glands contributing to high aldosterone levels often observed in obesity. Importantly, several studies have demonstrated prevention of weight gain and significant metabolic benefits after the use of mineralocorticoid receptor antagonists in animal models of genetic or diet-induced obesity. Despite these encouraging data, there are still few studies showing significant beneficial metabolic effects derived from the use of mineralocorticoid receptor antagonists in clinical settings. Therefore, planning well-structured clinical trials will be necessary to better elucidate the role and effectiveness of these drugs in obesity and metabolic syndrome in humans.

Keywords
Mineralocorticoid receptor, aldosterone, adipose tissue, adipocyte, browning, adrenal glands

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Introduction: Mineralocorticoid receptor (MR)

Mineralocorticoid receptor (MR, see *Table 1* for nonstandard abbreviations) is a member of the nuclear receptor superfamily and acts as a ligand-dependent transcription factor. Its activation by aldosterone - the main endogenous mineralocorticoid hormone - at the level of renal tubular epithelial cells represents a crucial mechanism for the regulation of salt and water homeostasis, plasma volume and blood pressure[1].

Nevertheless, MR also binds glucocorticoids with a 10-fold higher affinity than aldosterone itself. In epithelial tissues (e.g. kidney, colon) the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) catalyzes the conversion of cortisol to inactive metabolite cortisone, thus preventing the illicit activation of MR by glucocorticoids.

On the other hand, in non-epithelial tissues (e.g. heart, blood vessels, brain, and adipose tissue) MR is mainly activated by glucocorticoids, given the virtual lack of the aforementioned enzyme in such tissues[2]. During the last decade, the identification of MR in various non classical aldosterone-selective target tissues led researchers to investigate the extra-renal functions of this receptor. Recent studies have demonstrated an important role of MR in the regulation of adipose tissue physiology and pathophysiology. In fact, MR is involved in several processes within adipose tissue, including adipocyte differentiation, autophagy and adipokine secretion[3,4]. Moreover, an excessive adipose MR activation has been shown to trigger most of the pathological conditions typically observed in metabolic syndrome, such as oxidative stress, endothelial dysfunction, insulin resistance, and systemic inflammation[2,3].

11β-HSD2	11β-hydroxysteroid dehydrogenase 2
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BMI	Body mass index
CT	Computed Tomography
CTRP1	Complement-C1q TNF-related protein 1
CYP11B2	Aldosterone synthase
MCP-1	Monocyte chemoattractant protein-1
MR	Mineralocorticoid receptor
Myf5	Myogenic factor 5
PPARγ	Peroxisome proliferator-activated receptor-gamma
ROS	Reactive oxygen species
TNFα	Tumor necrosis factor alpha
UCP1	Uncoupling Protein 1
WAT	White adipose tissue

Table 1. *List of nonstandard abbreviations.*

The adipose organ

For several years adipose tissue has been considered as a metabolically inert triglycerides storage compartment, devoid of any anatomical organization. However, the wide array of studies conducted on adipose biology and function over the last decade helped to define the novel concept of "*adipose organ*"[5]: a multi-depot organ consisting of subcutaneous and visceral depots, with a remarkable cellular heterogeneity and anatomical complexity including a stromal vascular fraction, nerve fibers and different cell types, such as adipocytes, preadipocytes, endothelial cells and resident immune cells[6].

Different types of adipocytes

Adipocytes represent the main parenchymal cells of the adipose organ. In mammals, two different cell types have been extensively described, organized in two different tissues and characterized by distinct origin, morphology and function. White adipose tissue (WAT) contains white adipocytes, which are unilocular spherical cells with the nucleus squeezed to the cell periphery and a single large cytoplasmic lipid droplet occupying almost 90% of cell volume.

These cells display two essential functions: 1) energy storage for the metabolic needs of the organism in the form of triglycerides, 2) endocrine function consisting of synthesis and secretion of several secretory proteins called "adipokines" (e.g. leptin, adiponectin, resistin) involved in different physiological processes, including body weight homeostasis, appetite regulation, glucose and lipid metabolism[6,7]. Conversely, brown adipose tissue (BAT) is made up of multilocular cells with a round central nucleus and a large number of cytoplasmic lipid droplets and cristae-enriched mitochondria that contain the uncoupling protein 1 (UCP1).

The latter protein represents the hallmark of brown adipocytes and is essential for the thermogenic function of BAT, promoting energy expenditure, uncoupling oxidative phosphorylation from ATP synthesis and dissipating chemical energy as heat[6,8]. Moreover, white and brown adipocytes display a distinct embryonic origin: white adipocytes derive from myogenic factor 5 (myf5)-negative progenitors, whereas classical brown adipocytes originate from myf5-positive embryonic precursors[9]. Interestingly, a further type of adipocyte, called beige (or *brite* or *brown in white*), has been recently observed in murine WAT[10]. Beige adipocytes show intermediate features between white and brown adipocytes. In fact, they have a morphology similar to that of brown adipocytes (high amount of mitochondria and cytoplasmic lipid droplets) even if they appear to originate from the same embryonic precursors of white adipocytes (myf5-negative precursors)[11,12].

Of note, these cells appear in murine WAT in response to cold stimulus or β3-adrenergic agonists, thus showing increased UCP1 levels and thermogenic activity[13].

These peculiar properties of beige adipocytes could explain the high plasticity of the adipose organ, being responsible for the process known as "*browning*", consisting in the acquisition of BAT features by WAT[10].

Role of MR in regulation of adipose tissue physiology and pathophysiology

Since MR was identified in murine and human adipose tissue, much progress has been made in the comprehension of its role in adipocyte. In vitro studies demonstrated that MR expression increases during the course of adipocyte differentiation, even showing that both aldosterone and glucocorticoids promote preadipocyte differentiation into white adipocyte through specific MR activation[14].

Consistent with these findings, pharmacological blockade of MR exerts a potent antiadipogenic effect in murine and human preadipocytes by inhibiting adipose differentiation and decreasing the expression of *Peroxisome proliferator-activated receptor-gamma* (PPARγ)[15], the "master regulator" of adipogenesis in mammals[16]. It has been widely documented that in obese individuals impaired insulin sensitivity is associated with adipocyte hypertrophy and increased expression of pro-inflammatory adipokines, which in turn leads to progressive macrophage infiltration and chronic low-grade inflammation into adipose tissue[17-19]. Aldosterone increases expression of pro-inflammatory cytokines (e.g. TNFα, MCP-1) in adipose tissue. Accordingly, MR antagonism reduces the levels of pro-inflammatory cytokines and reactive oxygen species (ROS), suppresses macrophage infiltration and increases expression of adiponectin in adipose tissue of obese mice[20,21]. Moreover, MR blockade curbs impairment in glucose tolerance and prevents body weight gain and white fat expansion in mice fed a high-fat diet[4]. The obesogenic role of MR has been further confirmed by Urbanet et al., identifying a transgenic mouse model in which the upregulation of adipocyte MR has led to the development of some of the metabolic syndrome features, including increased body weight and visceral fat mass, insulin resistance and high levels of triglycerides[22]. Furthermore, recent evidence supports the existence of a novel endocrine axis between adrenal glands and adipose tissue, which may explain high levels of aldosterone frequently observed in obesity even in patients under treatment with either angiotensin-converting enzyme inhibitors or angiotensin receptor blockers[23,24]. Specifically, adipose tissue releases into the bloodstream various secretory products (e.g. CTRP1, leptin) which can directly stimulate adrenal glands to synthesize and secrete aldosterone by increasing the expression of aldosterone synthase (or CYP11B2), regardless of the renin–angiotensin system[25,26].

In turn, aldosterone binds and activates adipocyte MR, thus promoting adipose expansion, chronic inflammation, oxidative stress and further production of adrenal-stimulating factors by adipose tissue, resulting in a detrimental vicious cycle between adrenals and fat[27]. Finally, another intriguing aspect to be elucidated is the role of MR in browning of WAT. In mice fed a high-fat diet MR antagonism has been shown to promote browning of WAT by reducing adipocyte autophagic rate both in vitro and in vivo, with a parallel increase in UCP1 levels in visceral and inguinal fat depots[4].

The main detrimental effects of MR activation on adipose tissue function and metabolism are summarized in *Figure 1*.

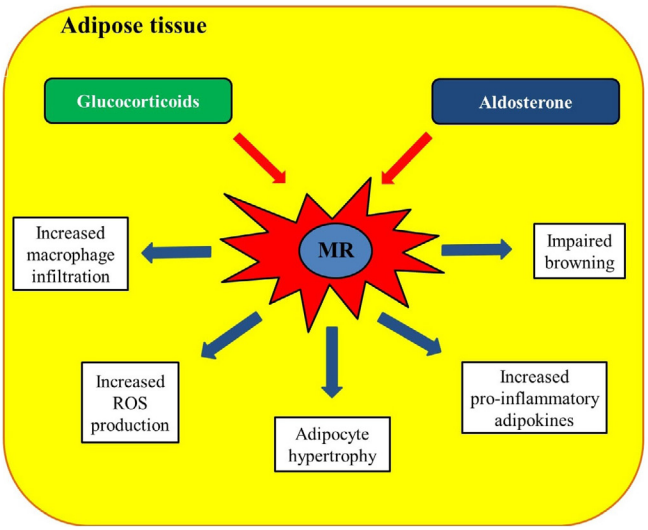

Figure 1 *- MR activation in adipose tissue is sustained by both aldosterone and glucocorticoids, given the lack of 11β-HSD2 in such tissue. This leads to a multitude of detrimental effects on adipose tissue function and metabolism, such as adipocyte hypertrophy, increased macrophage infiltration, upregulated expression of pro-inflammatory adipokines, increased oxidative stress through augmented ROS production, and impaired browning process. Abbreviations: MR, mineralocorticoid receptor; ROS, reactive oxygen species.*

Potential use of MR antagonists in obesity and metabolic syndrome

Although several studies on murine models with genetic and diet-induced obesity clearly demonstrated beneficial effects of MR antagonism in terms of metabolic outcomes (body weight, fat mass, adipose tissue inflammation, insulin sensitivity and lipid metabolism), there are still few data on the potential use of MR antagonists for prevention and treatment of obesity and metabolic syndrome in humans. Karashima et al. recently showed that in patients with primary aldosteronism the mineralocorticoid receptor antagonists spironolactone and eplerenone significantly reduced body mass index (BMI) and visceral fat area after 12 months of treatment[28]. Another study demonstrated that surgery or spironolactone rapidly and persistently restores normal sensitivity to insulin in patients with primary aldosteronism[29]. However, more studies and clinical trials are necessary to strongly confirm these promising findings.

Conclusions

During the last decade the detrimental consequences of MR activation in adipose tissue have been clearly demonstrated. In fact, MR blockade has been shown to prevent fat mass expansion, impairment in glucose tolerance and adipose pro-inflammatory status in murine models with obesity. Moreover, MR antagonism promotes the development of beige adipocytes in murine WAT (process known as "*browning*"), thus reducing the adverse effects of WAT expansion and inflammation. In light of these data, the potential use of MR antagonists for prevention and treatment of obesity and metabolic syndrome in humans represents an intriguing field of research. However, despite the wide use of MR antagonists in patients with heart failure

and left ventricular dysfunction after myocardial infarction[30], evidence of beneficial metabolic effects of MR antagonism in adipose tissue has been definitely documented only in animal models[4,15,20,21]. Nonetheless, few encouraging data show improved metabolic health by using MR antagonists in patients with primary aldosteronism, even if more appropriate clinical trials are needed in the future to better elucidate the real benefit of these drugs in patients with obesity and metabolic syndrome.

Disclosure Statement

The authors declare that they have no conflict of interest.

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aesthetic medicine

Review

Gender-specific development and distribution of adipose tissue depots: a literature review

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Abstract
Remarkable differences exist between males and females in the development and distribution of adipose tissue depots. This gender dimorphism is essentially related to the White Adipose Tissue (both visceral and subcutaneous) since males mainly have an android pattern of distribution of body fat ("apple shaped") while women have a gynoid pattern ("pear shaped"). This different adipose tissue localization, as well as total body fat, is now widely recognized as risk factor for metabolic diseases. This review is aimed at describing the main health-related implications of this gender dimorphism and the mechanisms underlying its development.

Keywords
Adipose tissue, body fat distribution, abdominal fat, sex steroid hormones, lipid metabolism

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Introduction

Significant differences exist in development, quantity and distribution of adipose tissue between men and women, widely documented by scientific literature[1,2].

The first substantial difference between males and females is represented by the total amount of body fat[3-5]: women, at equal Body Mass Index (BMI), ethnicity and age, have about 10-15% body fat more than men[2,5-7]. Indeed, as reported in literature, healthy sedentary or moderately active women with a BMI of 20-25 kg/m^2 often have a 25-35% of body fat, while men with the same BMI have 12-20% of body fat[6,7].

This is partly due to the amount of primary or Essential Fat (EF). Indeed, although the proportional amount of average fat storage is similar, accounting for about 12% and 15% of body weight in men and women respectively, the amount of EF in females is four times higher than in males. EF in women represents 12% vs 4% in men of the total adipose tissue, due to the presence of gender-specific depots, accounting for about 5-9% of total body fat, in breasts, genital regions, lower body and intramuscular level. These depots, facing the need of an increased energetic demand at certain stages of life, such as pregnancy, support normal reproductive functions[8].

In addition to the total amount of adipose tissue, however, even more remarkable differences are found in its different distribution in body districts of men and women[1-6,8,9].

This gender dimorphism is essentially related to the White Adipose Tissue (WAT). In fact, men and women seem to have a similar regional distribution of Brown Adipose Tissue (BAT), although imaging studies through PET-TC have shown that some differences are visible also for BAT, because it is more prominent in the cervical-supraclavicular region in females compared to males, with a ratio of 2:1[2].

Given these presuppositions, the first consideration to be made is that the amount of total body fat as well as its distribution have a meaning that goes far beyond the mere aesthetic interest, since it is now widely recognized that both total fat and its distribution affect systemic metabolism, acting as risk factors for metabolic diseases[3]. Therefore, this literature review was aimed at investigating the main health implications of the gender-specific distribution of adipose tissue, as well as the main mechanisms underlying the different development and distribution of fat depots between men and women. In order to achieve our goal, we considered the most recent papers published on this topic.

Gender dimorphism in the distribution of WAT and the associated different metabolic risk pattern

Although BMI and total fat surplus are associated with the onset of several comorbidities and represent a strong predictor of overall mortality, localization itself of adipose tissue depots in our body is a major predictor of the risk of metabolic disease. Several studies have shown the correlation between WAT depots and metabolic risk in humans, probably due to the intrinsic differences in the activity of adipose tissue, which is no longer considered as a mere lipid depot but as an active organ with its own functions[1,3,4,9].

The two types of WAT, visceral (VAT) and subcutaneous (SAT), are defined by their localization: the former is usually represented by the omental depot while the latter by the abdominal, gluteal and femoral depots[1,6].

As already said, the main differences in the distribution of adipose tissue in men and women are related to WAT localization and, more in detail, substantial differences are due to the different distribution of VAT and SAT between genders[2,4-6,8,9]. In fact, while males have, in principle, an android pattern of distribution of body fat ("apple shaped") with a greater VAT depot in the upper body, women have a gynoid pattern ("pear shaped"), characterized by a localized SAT depot in the lower body[2,6,8,9]. Therefore, females, in addition to a higher percentage of total body fat, have a greater amount of SAT, both in the abdominal region as well in the gluteus-femoral one[2,4-6,8-10]. On the other hand, their amount of VAT is lower, given that in lean men VAT accounts for about 10% of the total fat mass, while in women it is about 5%[6]. Then, women usually store smaller amounts of adipose tissue in the abdominal region, particularly VAT, unlike men in whom 98% of abdominal fat is stored exactly at visceral level[2].

Another significant difference between men and women is the way the adipose tissue expands: this can be achieved by adipocytes growth or progenitors recruiting. Gender differences in fat distribution, therefore, involve both cells size as well as their number. In females, gluteo-femoral adipocytes are larger than those of men, abdominal ones are similar and visceral ones are smaller. Nevertheless, the increase of subcutaneous depots in obese women is mainly due to the increase of the number of cells, rather than their dimensions. Indeed, through imaging techniques it has been observed that femoral fat accumulation in women is associated with hyperplasia of adipocytes, while in men with hypertrophy of the same. Neverthless, fat accumulation in the subcutaneous abdominal area is associated with hypertrophy in both genders but since women have a greater number of adipocytes even in the lean state, they may accumulate more fat mass[5].

Dimorphism in the localization of adipose tissue, as described herein, has remarkable functional implications as it affects adipokine production, insulin sensitivity growth process, certain inflammatory responses, mitochondrial function, lipolysis, and free fatty acids release[10]. The different distribution of body fat depots, therefore, has implications for the individual metabolic risk profile, in fact it is known that central fat accumulation (both subcutaneous and visceral) is associated with increased susceptibility

to metabolic disease. Several studies have confirmed the relationship between central adiposity and risk of diabetes, cardiovascular events, hypertension, sleep apnea, cancer and global mortality rates[1,7]. The different risk pattern in relation to the distribution of body fat is also supported by the evidence that men and women in the highest quintile of waist circumference have a mortality rate almost double that of those in lower quintiles[7]. Conversely, the gluteo-femoral adipose tissue, and hence the gynoid conformation, is associated with a better metabolic profile, regardless of the amount of total adipose tissue, and it can be protective against the adverse effects of obesity, both in males and in females[1,2,5].

The role of sexual steroid hormones in the different distribution of adipose tissue depots

The differences in the localization of adipose tissue depots, influenced not only by gender but even by ethnic origin, are probably due to a complex interaction of genetic, epigenetic and hormonal factors[5,10]. On the basis of the available scientific evidence, it is now considered that gender differences in body proportions and body fat distribution are mainly related to differences in endocrine status (estrogen, androgens, GH, IGF-1) emerging from prepuberal phase and diminishing with menopause[1,4-7]. However, very little is known about the cellular and molecular mechanisms through which sex steroids modulate growth, metabolism and size of specific fat depots in humans[5].

The involvement of sex hormones is suggested by the evidence that the extent of differences in fat depots between males and females is boosted with the growth and especially from late puberty until early adulthood, when men acquire a physical conformation of android type while women of gynoid type[8]. It is known that sex hormones have several effects on body fat, indeed they are endogenous modulators of both development and activity of adipose tissue and may affect the distribution of SAT and VAT depots[1,5,7]. Nevertheless, the exact cellular and molecular mechanisms underlying this regulation remain largely unclear[1,5]. What is known is that human adipocytes, as well as preadipocytes, express receptors, both in SAT and in VAT, for sexual steroids[1,5] and in particular estrogens receptors (ERα, ERβ and its variants as well G protein-coupled estrogen receptors) and androgens receptors (AR). According to some evidence, there are also regional and gender differences in the expression of these receptors. Specifically, the expression of ERβ1 (both mRNA and protein) seems to be much lower in intra-abdominal VAT compared to SAT. Regarding ERβ4 and ERβ5, their mRNA levels are significantly higher in the gluteal SAT than in the abdominal one, and this seems to be true in males as well as in females. Overall, ERβ mRNA levels are shown to be higher in women than in men[1]. In contrast to ERβ, ERα appears more pronounced in abdominal SAT than in gluteus SAT [1,11]. This differential expression of ER seems to be involved in the distribution of adipose tissue depots. Supporting this theory is the negative correlation between waist-to-hip ratio with the abundance of gluteal ERβ protein versus its positive correlation with the ERα / ERβ ratio. Moreover, ERα and ERβ are shown to mediate different actions for specific fat depots: *in vitro*, estrogens upregulate ERα and ERβ mRNA expression in female subcutaneous adipocytes but, as for men, this upregulation regards only ERα in subcutaneous and visceral adipocytes. Estrogens, therefore, could modulate fat storage in a storage-specific way through differential ER expression in WAT[1] and this receptor mechanism also appears to be involved in the changes occurring in women with menopause. In addition to the different expression of ER in adipose tissue, to support the involvement of sex steroids in determining differences in body composition there is the evidence that changes in body fat distribution occur in certain stages of life concurrently with the occurrence of changes in sexual hormones. Indeed, the main differences between men and women can be observed even before puberty, but they become much more pronounced after this period of life when gender differences emerge in the endocrine profile[5,6,8,10].

During puberty, in fact, the increases in levels of estradiol in girls are associated with the development of a gynoid pattern of fat distribution and with continuous increase in body fat, both in an absolute value as well as in percentage[5,6,10]. Nevertheless, in males, during puberty, the increase in testosterone serum concentration is associated with a percentage reduction of fat mass and with a relative increase in abdominal subcutaneous fat and, in general, scientific evidence indicates that increased concentrations of testosterone in men leads to a reduction of total fat mass. During puberty, therefore, while body weight gain in boys is mainly due to the increase of lean mass, in girls it is mainly due to the increase of fat mass[5,6].

Moreover, beyond the distinction between males and females, differences in the adipose tissue distribution, related to the hormonal profile, can also be observed right among women. In a study that analyzed plasma of girls in pre-puberty, it was observed that the distribution of body fat, rather than its amount, is associated with the total concentrations of 17β-estradiol (E2) and testosterone. Therefore, girls with fat depots mainly at hips level had higher levels of sexual steroids and gonadotrophin as a result of their high ovarian activity[8].

Another proof supporting the role of sex steroids derives from the finding that the effects of estrogens on body fat distribution are more clearly observed in trans-sexual subjects, where estrogenic treatment increases total fat and, especially, subcutaneous fat in the lower body[6].

As already mentioned, gender differences in the distribution of adipose tissue depots decrease with menopause, which is followed by a redistribution of the adipose tissue toward a more androgenic phenotype. During the peri-menopausal transition, in fact, there is an accumulation of adipose tissue at the visceral level, presumably because of the decline in estrogen levels. Therefore, postmenopausal women have a much greater increase in the volume of VAT than premenopausal ones[1,2,5,6,8], resulting in increased risk of cardiovascular diseases and metabolic syndrome[9].

To explain the change in the distribution of adipose tissue depots after menopause, two major mechanisms

have been suggested. First, the influence of E2 on adrenergic receptors modifies the characteristics of lipid storage in fat depots, since it can shift, within subcutaneous and visceral deposits, the balance between lipolytic receptors β1-2 and α2-adrenergic anti-lipolytic receptors. The altered distribution of α2 receptors and β receptors in adipose depots is involved in the modulation of distribution of fat in the different depots. Males have less anti-lipolytic receptors in their visceral depots, storing more fat in this location. Thus, in premenopausal women, a higher anti-lipolytic α2/lipolytic β ratio in visceral depots may limit fat accumulation. Another theory suggests that, after menopause, adipose tissue becomes the primary source of E2, and the conversion process of E2-precursors into E2 by aromatase may occur mainly in visceral depots. This depot, therefore, could increase in an attempt to overcome, at least in part, the lack of E2 in menopause[9]. In addition to estrogen, also androgens may have depots- and gender-specific effects on the distribution of adipose tissue[1]. Indeed, visceral adiposity increases in men with aging, as well as in women, and this seems to be linked to the reduction of testosterone with age[1,5,6]. In support of this theory, testosterone therapy in older men seems to reduce the visceral fat mass and increase lean muscle mass. In women, however, the levels of testosterone are correlated with a significant increase in abdominal fat, so much so that in women with polycystic ovarian syndrome, often characterized by a hyperandrogenic state, there is an increase of the VAT[1]. In summary, the role of steroid hormones in gender dimorphism of body fat seems to be primarily supported by the fact that differences in the development and localization of adipose tissue depots are preceded by variations in the production of sexual steroids. Hence, these differences emerge and become evident during puberty. Secondly, menopause, due to the change in endocrine status, is characterized by significant changes in energy metabolism that reduce gender differences through a transition to central obesity. Finally, also the reduction of testosterone, with age, in men leads to increased visceral fat[2,5,6,8,10].

Differences in lipid metabolism as a contribution to gender dimorphism in the distribution of adipose tissue depots

In addition to what has been discussed so far, regulation of lipolysis of the local adipose tissue could be an important factor contributing to differences in body fat gender-specific distribution. In fact, it has been noticed that there are significant differences in lipid metabolism between men and women, especially regarding mobilization and oxidation of fatty acids[4,8,10]. Indeed, although subcutaneous fat in the upper body has a greater lipolytic activity than the lower body, both in males and females, women tend to accumulate free fatty acids while men tend to oxidize them more easily. In addition, men are also less sensitive to the anti-lipolytic effects of insulin. These differences presumably reflect the opposite gender-specific specialty in energy utilization since higher insulin sensitivity and lower muscle mass observed in women preferentially lead to energy storage in WAT rather than to its oxidation[4]. This tendency for a higher accumulation of lipids in women could, as has already been mentioned, be functional to the increased energy demand associated with reproduction[8].

Sexual steroids seem to be involved even in these differences in lipid metabolism, as both estrogens and androgens regulate lipolytic responses to catecholamines through, at least in part, changes in the expression of adrenergic receptors[5]. *In vitro* studies, performed on adipose tissue biopsies, indicate that differences in catecholamines-mediated lipolysis between fat depots in upper and lower body are more pronounced in women than in men. Similarly, it has been observed that lipolytic effect of noradrenaline is stronger in abdominal adipocytes than in the gluteal area and this regional difference seems more pronounced in females than in males. In addition, although α2-adrenoceptor density is almost homogeneous in all adipose depots, female affinity for clonidine, a specific α2-adrenergic agonist, appears to be 10 to 15 times lower in abdominal adipocytes than in the gluteal area. Hence, based on these findings, it seems that in women the lower body fat lipolysis is less sensitive to β-adrenergic stimulation than that in the upper body. Moreover, it appears that regional differences in catecholamines-induced lipolysis are related to site-specific variations in β-adrenoceptors density and that variations in α2-adrenergic receptors affinity explain, at least in part, the greater lipolytic response induced by catecholamines in women[8]. It is important to point out, however, that the mere regional differences in lipid metabolism do not fully explain the gender dimorphism observed in the distribution of body fat[4,6,7], although a general trend in regional lipid utilization is consistent with android and gynoid conformations observed in males and females, respectively[4].

Conclusions

In conclusion, it is clear that underlying the different distribution of adipose tissue depots there is a complex network of biological and behavioural issues. Therefore, the interaction between several factors and mechanisms contributes to inducing and consolidating differences in storage and localization of adipose tissue between men and women, as well as the underlying differences in substrate metabolism[8].

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aesthetic medicine

Original Article

In-vivo injection of oligonutrients and high molecular weight hyaluronic acid - results of a randomized controlled trial

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Abstract

Introduction: clinical efficacy for skin rejuvenation and the safety of a commercially available Dermo-Restructuring Complex with 0.3% lidocaine, composed of natural high molecular weight hyaluronic acid (15 mg/mL) and 14 selected bioactive compounds have been assessed through an interventional, monocentric, randomized, double blind, split-face comparative study.

Subjects and methods: the study was designed to evaluate the efficacy and safety of repeated microinjections of the product *versus* a physiological saline solution. A total of 25 volunteers aged over 40 years were enrolled. Three sessions of injections at 3 weeks intervals were performed on the face and neck. Skin roughness, relief, thickness, elasticity, hydration and radiance were assessed before and then 2 weeks as well as 3 months after the last injection. Patient satisfaction to treatment was also evaluated.

Results: at 2 weeks after the last injection, the primary criterion (roughness on the cheeks) was significantly improved: a significant effect of the tested product was indeed demonstrated on skin moisture, roughness of the cheeks in comparison with physiological saline solution injection. Skin elasticity and radiance were also improved 3 months after the end of treatment. Patient satisfaction was higher for the treated group. This trial demonstrated the benefit of repeated microinjections of the Dermo-Restructuring Complex with lidocaine to restore the quality of young skin. This study also suggests that this treatment can prevent premature ageing of the skin.

Keywords

Hyaluronic Acid, oligonutrients, skin quality, biometrology, double-blind randomized study, skin rejuvenation

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Introduction

With ageing, as well as with the individual impact of intrinsic (genes, hormones, diseases, etc.) and extrinsic factors (environmental factors such as sun exposure and pollution, diet, drugs, smoking, etc.), the skin is subject to morphologic and mechanical changes that lead to wrinkles as well as to skin laxity, dehydration and loss of elasticity. Nowadays a better knowledge of skin metabolism and architecture reveals that the alterations of the dermal connective tissue mainly correspond to an age-related loss of the extracellular matrix (ECM) and its components: fibroblasts, collagen, elastic fibers and hyaluronic acid (HA)[1,2,3]. The ageing process not only reduces the ECM density but also affects its quality[4]. Indeed, the reduction of the ECM components is responsible for the deep modifications in the mechanical properties of the skin. Moreover, the reduced activity of fibroblasts makes the newly synthetized collagen more easily degraded by collagenases and metalloproteinases, the key enzymes in matrix turnover. HA plays a crucial role in skin integrity and ageing since its amount decreases with age as well as with the expression of the major cell surface hyaluronate receptor, CD44[1,3].

The increasing concern and demand to maintain a youthful appearance have led to the emergence of dermatological procedures for the treatment of skin ageing. Among them, minimally invasive treatments such as mesotherapy, mesolift or biorejuvenation have been extensively used to treat skin volume loss, wrinkles and photodamage[4]. These procedures consist of superficial microinjections of mixtures of bioactive and readily absorbable bioactive substances (such as multivitamin solutions) and are able to promote skin rejuvenation[2,3,4,5]. The desired effect is to rebuild a hydrated, firm, radiant and elastic skin. Several studies concluded that dermal multi-injections of natural HA alone or combined with natural small biomolecules (e.g. mannitol, glycerol, cocktails of multivitamins) are generally safe and well appreciated by patients although, usually, clinical efficacy has not been demonstrated. The results suggest that the treatments are successful in delivering new HA into the skin with the effect of improving skin hydration, firmness and elasticity without any unacceptable damage or negative change of the skin structure[2,3,6,7,8]. However, to date, except for the randomized controlled study published by Baspeyras et al.[2], objective data supported by instrumental evaluations are very sparse and/or not based on proper biometrologic assessments of the skin which could definitely confirm the benefits of a particular formulation.

The main objective of this study was to assess, on scientific and methodological bases, the safety and efficacy of a commercially available Dermo-Restructuring Complex with lidocaine (DRC) for skin biorejunenation.

The DRC is a readily injectable solution composed of natural high molecular weight HA (15 mg/mL) and 14 selected bioactive compounds. Lidocaine (0.3%) is added to the DRC formulation to reduce the pain during injection. The biorejuvenation protocol with DRC consists of three sessions of intradermal microinjections in the face and neck at three weeks intervals. We report a direct comparison of the injection of DRC *versus* a placebo control (saline solution) in a randomized split-face design. Evaluations were done both on the face and the neck. Changes in skin roughness, thickness, hydration, elasticity, radiance between the two groups have been observed as well as patients' satisfaction and product's safety profile.

Finally, this is the first report on the safety and efficacy of DRC for skin rejuvenation.

Methodology

An interventional, monocentric, randomized, double-blind, comparative study aimed at evaluating the effect of the DRC vs. physiological saline solution injections on skin quality, and biometrological parameters. The study was carried out at the Centre for Study and Research on Integuments (CERT), department of dermatology, university hospital of Besançon in France. All steps taken during the research were conducted in compliance with the protocol and the principles of the Helsinki declaration (amended in Tokyo, Venice, Hong Kong, Somerset West, Edinburgh) and also with local laws and regulations governing clinical trials and Good Clinical Practice (ICH E6 + decision of November 24th, 2006 published in the official Journal of November 30th, 2006).

The protocol was approved by the institutional review board "Comité de Protection des Personnes CPP Est II" (Besançon, France), on June 17th, 2013 and the French national agency for drug safety ("Agence nationale de sécurité du médicament et des produits de santé", ANSM) on June 25th, 2013. Each volunteer signed a written informed consent.

Participants

25 volunteers (22 women and 3 men) aged over 40 years with Fitzpatrick phototypes II to IV (plus one subject with Fitzpatrick phototype V) who presented a photoageing score of 2 to 3 on the Glogau scale were included in the study. Mean age was 55.7 ± 8.9 years [range: 41-70 years]. The exclusion criteria were: tobacco consumption more than 10 pack-years, laser treatments for skin rejuvenation or laser lifting in the last year, or surgical lifting in the 2 years prior to the study, a history of filler injections (HA-type) during the last year, non-permanent to semi-permanent injectable implants in the 2 years prior to the study, facial skin support devices (mesh, gold thread, etc.), peeling or ultrasound performed in the last six months, known hypersensitivity to HA with a history of severe allergies or anaphylactic shock, known hypersensitivity to lidocaine and/or amide-type local anesthetics, hypersensitivity to one of the DRC's excipients, application of a whitening product on the areas of interest in the month preceding the study, tendency to develop inflammatory skin conditions or hypertrophic scars, wound healing or coagulation disorders, presence of foreign bodies such as liquid silicone or other synthetic particles, aspirin or anti-inflammatory drug intake 15 days prior to the procedure, exposure

to the sun or to UV tanning radiation in the last 15 days, skin disorder or inflammation in the study zone and surrounding area, any general or skin pathology, dermatosis, autoimmune disease, systemic chronic or acute disorder and any topical or systemic treatment which could interfere with the treatment or influence the study results. Female volunteers were excluded if they were pregnant (or planning a pregnancy during the study), breastfeeding or presenting a positive test for the HCG pregnancy test.

Study product

The active product DRC is CE-marked and has been available in Europe since 2011 under the commercial name of TEOSYAL® Redensity [I] (TEOXANE Laboratories, Switzerland). It consists of a sterile, non-pyrogenic injectable solution of 15 mg/mL of non-animal high molecular weight HA supplemented with 14 bioactive oligonutrients and 0.3% of lidocaine. The oligonutrients are selected compounds, all of pharmaceutical grade: 8 amino-acids (arginine, glycine, leucine, isoleucine, valine, lysine, threonine and proline), 3 anti-oxidants (glutathione, alpha-lipoic acid and N-acetyl-L-cysteine), 2 minerals (zinc acetate and copper sulfate) and vitamin B6. The DRC product was provided in boxes including two blisters, each with a pre-filled sterile 1mL syringe supplied with 2 x 30G ½ inch needles.
Physiological saline solution (sodium chloride PROAMP 0.9%, Laboratoire AGUETTANT, France) was used as the control product.
The study consisted of 3 injections at 3 weeks intervals and 2 follow-up visits, the first one just before the first injection, the second 2 weeks after the last injection session and the third 12 weeks after the last injection session. During each treatment session, the subject received about 1mL of study product in one side of the face, 1mL of study product in one side of the neck and the same amount of control product in the other side of both face and neck, to overcome the inter-individual variations in skin status. The same trained investigator administered HA and control products to all subjects. Both products were injected manually into one side of the cheek and the neck, at the level of the superficial dermis using multiple microinjections.
Then the sites of injections received gentle massage from the investigator to ensure optimal product absorption.

Study design and protocol

Three injection sessions were performed at 3 weeks intervals (D0, W3, W6) and assessments were made before injection (D0), two weeks after the last treatment session (W8) and 3 months after the last injection (W18).
The study was double blind, the volunteers and the investigators in charge of the evaluations were blinded to the treatment; only the treating investigator was not blinded to the treatments (DRC or control). The treatment side was allocated according to a randomization list.
At the injections visits, the treating investigator was required to thoroughly disinfect the areas to be treated using 0.05% sterile coloured aqueous chlorhexidine.
No topical anesthetic was used pre-injection. Injections were made using 30G ½ inch needles. Products were injected evenly on the areas to be treated, using the serial puncture technique, making a visible bolus of two to three millimeters in diameter (containing almost 0.05 mL) into the reticular dermis or the mid dermis. The micropunctures did not need to form any large, visible papules. The total volume injected was 1mL per half-face and 1 mL per half-neck.
Before injection, patients were asked to avoid sun or artificial UV exposure (15 days prior to the injection) as well as saunas, contact sports, alcohol (the day before injection) and vitamin E in high doses, aspirin, anti-inflammatory or anticoagulants drugs (the week before the procedure). Throughout the study period no change to facial hygiene products or routine was allowed nor were self-tanning applications.
No application of any medicinal product during the study without notifying the investigator was permitted. UV ray and sun exposure was not allowed throughout the period. The subjects were asked to avoid the application of any make-up during the next 12 hours and any intensive facial treatment, extreme temperature and contact sports for one week after injection.

Evaluation criteria & Assessments

Study zones and area of analysis on the cheeks and the neck are presented in *Figure 1*.

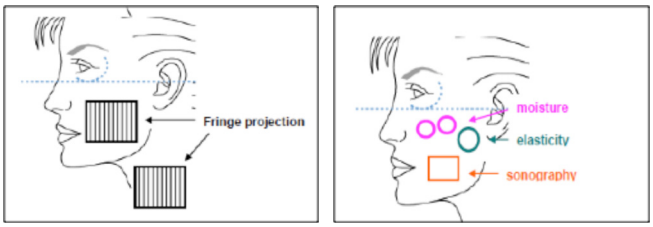

***Figure 1**. Techniques used and areas analyzed in the study.
All measurements were taken on the face (cheeks) and some on the neck.*

Primary endpoint:

The primary endpoint was the evolution from D0 of the relief of the cheeks at W8. Success was defined by a p value of the test between groups <0.05. The relief in the cheek area (Sa, roughness parameter) was evaluated by fringe projection at the D0, W8 and W18 time points. Fringe projection uses the principle of interferometry. The method consists in projecting a network of fringes onto the area to be analyzed. Fringes are deformed proportionally to relief. Making four separate acquisitions enables the software to reconstitute the 3D profile of the explored area by calculating the height of each point. The instrument used was the DermaTop® manufactured by Breuckmann GmbH (Eotech). This instrument consists of a measurement sensor with a LED projector which emits the fringes, and a CCD camera, calibrated to a defined measurement field. A 100 fold magnification lens was used for the study, corresponding to a field of view of 60 x 80 mm with 4 cm depth.

Secondary endpoints:

- Relief (Sa, roughness parameters) was evaluated in the

neck area by fringe projection at D0, W8 and W18 time points.
- Dermal thickness using DermCup high frequency (20MHz) ultrasound was measured in order to monitor the evolution with time.
- Skin hydration using Moisturemeter D was measured on the cheeks at D0, W8 and W18.The depth of measurement was 1.5mm (S15 probe). The measured value is proportional to the water content.
- Skin elasticity measurements were obtained using a Ballistometer® at D0, W8 and W18 on the cheeks. The principle of ballistometry relies on the use of a mass causing an impact on the surface of the skin in order to measure its mechanical properties[9,10]: different parameters were assessed: the indentation which represents a direct measure of the firmness of the skin (the greater the value, the more "flaccid" the skin is), the alpha value which, if high, points to poor elasticity and finally the coefficient of restitution (CoR) that reports a more elastic skin if the value is close to 1.
- Evaluation of skin radiance using the C.L.T.B. scoring method was performed by a trained, blinded investigator at D0, W8 and W18[11].
- Self-assessment by the patients was also recorded using a standard questionnaire for face and neck.
- Common and local treatment reactions were recorded by the subjects every day using a daily diary until W8 visit. The aim was to assess the presence and the severity (mild, moderate or severe) of redness, pain, induration, swelling/edema, unevenness, haematoma, itching, discoloration/coloration or any other adverse effect on each side of the cheeks and neck.
- At each visit, any localized intolerances (erythema, desquamation, tingling, burning or other) or any adverse event were also recorded by the treating investigator.

Statistical analyses:

The statistical analysis was performed after database lock. The continuous variables were described as number of subjects, mean, standard deviation, minimum, maximum, median and quartile. Qualitative variables were presented as numbers and as percentages. A normality test was performed on the data. A statistical comparison of baseline values (continuous variables) was performed: in the case of a normal distribution, the paired Student t test was used to compare baseline values; in other cases, the paired Wilcoxon test was used. The same was done to compare post-treatment values (continuous variables). Finally, evolution between time points and treatments was analyzed. A repeated measures analysis of variance on both factors (treatment, time points) in case of normal distributions was done. In case of non-normal distributions, a paired Wilcoxon test and a Friedman test (to analyze remanence) were performed on deltas (treated –non treated). The significance level was set to 5% and the trend level to 10%.

Results

Primary efficacy criterion: Effect of DRC and control on skin roughness in the cheek area

At D0, skin roughness parameter was not significantly different between control and DRC-treated cheek area *(Table 1)*. Between D0 andW8, *Sa* parameter significantly improved by decreasing 5.9% in the DRC-treated hemi-faces (p =0.0393), whereas it was non-significantly increased in the control hemi-face (+1.4%, p = NS) *(Table 1)*. Moreover, this evolution of the DRC-treated group was significantly different from the evolution in the control group (p = 0.0011), which validates the primary efficacy criterion of the study. Three months after the end of the treatments (W18 post baseline), no significant difference persisted anymore in the DRC-treated group (+0.4%, p = NS), while a significant worsening of skin roughness was observed in the control group (+6.6%, p = 0.0020) *(Table 1)*. The evolutions from baseline were statistically different as well between the two groups at W18 (p = 0.0409).

Cheek roughness *Sa*, µm (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
DRC	50.38 ± 13.41	47.43 ± 11.62	- 5.9*	50.60 ± 15.09	+ 0.4
Control	48.83 ± 15.03	49.50 ± 13.75	+ 1.4	52.06 ± 16.33	+ 6.6**
p value†	-	-	0.0011	-	0.0409

* Comparison *versus* D0, $p < 0.05$, Wilcoxon test

** Comparison *versus* D0, $p < 0.01$, Wilcoxon test

† Comparison of changes from D0 with DRC *versus* Control, Wilcoxon test

Table 1*. Skin roughness parameter Sa measured on the cheeks before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population, measured by fringe projection.*

Secondary efficacy criteria: Effect of DRC and control on skin roughness in the neck area

At D0, skin roughness parameter was not significantly different between control and DRC-treated neck area *(Table 2)*. At two weeks after the end of the treatments (W8 post baseline), changes from pre-treatment tended to show an improvement of the Sa parameter in the DRC-group and a worsening in the control group, but both evolutions were not statistically significant.

However the comparison between both evolutions showed a trend in favor of the DRC-treated neck sides ($p = 0.0973$) *(Table 2)*.

Three months after the end of the treatments (W18 post baseline), the change from pre-treatment status showed a slightly significant worsening of Sa parameter in both groups *(Table 2)*.

Effect of DRC and control on dermis thickness

Comparing to the pre-treatment status, the dermal thickness significantly increased either 2 weeks (+2.8%, $p = 0.0214$) or 3 months (+4.1%, $p = 0.0249$) after treatments in the DRC- treated cheeks *(Table 3)*.

The control side also revealed a significant increase in the dermal thickness at both time points (+4.2, $p = 0.0059$ and +4.2, $p = 0.0125$). No significant difference was observed between DRC and control treatments.

Neck roughness Sa, µm (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
DRC	54.08 ± 20.61	51.98 ± 18.29	- 3.9	55.61 ± 20.91	+ 2.8*
Control	51.40 ± 17.34	51.86 ± 17.80	+ 0.9	54.27 ± 17.92	+ 5.6**
p value†	-	-	0.0973	-	NS

* Comparison *versus* D0, slightly significant difference, $p = 0.0973$, Wilcoxon test

** Comparison *versus* D0, slightly significant difference, $p = 0.0759$, Wilcoxon test

† Comparison of changes from D0 with DRC *versus* Control, Wilcoxon test

Table 2. *Skin roughness parameter Sa measured on the neck before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population, measured by fringe projection.*

Dermis thickness, mm (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
HA product	1.45 ± 0.29	1.49 ± 0.22	+ 2.8*	1.51 ± 0.25	+ 4.1*
Control	1.44 ± 0.25	1.50 ± 0.23	+ 4.2**	1.50 ± 0.27	+ 4.2*
p value†	-	-	NS	-	NS

* Comparison *versus* D0, $p < 0.05$, Wilcoxon test

** Comparison *versus* D0, $p < 0.01$, Wilcoxon test

† Comparison of changes from D0 with DRC *versus* Control, Wilcoxon test

Table 3. *Dermis thickness measured on the cheek before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population, measured by echography.*

Effect of DRC and control on skin hydration

Results of dermis hydration measured on the cheeks are shown in *Table 4*.

Two weeks after the last injection, hydration of the deep dermis (1.5 mm probe) is maintained in the cheeks of the DRC-group (+0.3%, p = NS) whereas hydration almost significantly decreased in the control-group (-4.2%, p = 0.0758). The difference between both evolutions was statistically significant and in favour of the DRC-treated group (p = 0.0187) *(Table 4)*. Three months after the last injection (W18, *Table 4*) differences with pre-treatment and between groups were not significant.

Effect of DRC and control on skin elasticity

Skin elasticity was assessed through the measurements of 3 parameters of Ballistometer® *(Table 5)*. Results showed a decrease of skin hardness (increase of indentation) which tended to be significant for both DRC and control groups two weeks after the last injection (+7.3%, p = 0.0788 and +7.8%, p = 0.0627, respectively). However this loss in skin hardness only persisted on the DRC-treated cheeks 3 months after injections (+7.9%, p = 0.0907). This effect was associated with a significant increase in skin elasticity only in the DRC-treated group at 3 months after the last injection, measured by the decrease of alpha parameter (- 14.2%, p = 0.0329) and the increase of the coefficient of restitution *CoR*, closer to the theoretical value of 1 (+3.4%, p = 0.0892).

Skin hydration, UA (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
DRC	31.39 ± 4.13	31.49 ± 3.30	+ 0.3	30.99 ± 3.49	- 1.3
Control	32.05 ± 4.47	30.69 ± 3.94	- 4.2*	31.04 ± 3.96	- 3.2
p value†	-	-	0.0187	-	NS

* Comparison *versus* D0, slightly significant difference, p = 0.0758, paired Student t test

† Comparison of changes from D0 with DRC *versus* Control, ANOVA

Table 4. *Dermis hydration measured on the cheek before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population, measured by MoistureMeter D (S15, 1.5 mm probe).*

Ballistometer® parameter (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
Indentation					
DRC	0.520 ± 0.100	0.558 ± 0.098	+ 7.3*	0.561 ± 0.097	+ 7.9*
Control	0.510 ± 0.092	0.550 ± 0.109	+ 7.8*	0.526 ± 0.095	+ 3.1
p value†	-	-	NS	-	NS
Alpha					
DRC	0.028 ± 0.010	0.026 ± 0.008	- 7.1	0.024 ± 0.008	- 14.3**
Control	0.029 ± 0.008	0.026 ± 0.008	- 10.3*	0.028 ± 0.011	- 3.4
p value†	-	-	NS	-	NS
CoR					
DRC	0.694 ± 0.057	0.705 ± 0.053	+ 1.6	0.718 ± 0.057	+ 3.5*
Control	0.685 ± 0.054	0.705 ± 0.064	+ 2.9*	0.697 ± 0.064	+ 1.8
p value†	-	-	NS	-	NS

* Comparison *versus* D0, slightly significant difference, $p < 0.1$, paired Student t test

** Comparison *versus* D0, $p < 0.05$, paired Student t test

† Comparison of changes from D0 with DRC *versus* Control, ANOVA

Table 5. *Skin elasticity parameters measured on the cheek before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population, measured by Ballistometer®.*

Clinical scoring of effect of DRC and control on skin complexion radiance

Table 6 presents the results of the sensory evaluation according to the C.L.B.T.TM method by a blinded trained assessor, according to Musnier et al. This methodology is based on the visual perception of colouring (pink red, olive, beige, light pink), luminosity, brightness and transparency. Regarding visual perception of colouring, a significant decrease of pink red color was observed in both groups 2 weeks after the last injection (-28.6%, p = 0.0033 and -24.7%, p = 0.0071), which persisted 3 months after the last injection (-31.2%, p = 0.0001 and -19.2%, p = 0.0124) with a difference between both evolutions in favour of the DRC-treated cheeks (p = 0.0137). A decrease in olive color was observed, which tended to be significant in both groups 2 weeks after the last injection, becoming statistically significant in the DRC-group 3 months after the last injection (-20.2%, p = 0.0001). A significant decrease in beige colour was observed in both groups (-33.3%, p = 0.0001 and - 31.4%, p = 0.0001 at W18), as well as a decrease in light pink, albeit lower in amplitude (- 10.2%, p = 0.0006 and -12.7%, p = 0.0001 at W18). Globally, 3 months after injection, in the DRC-treated group, the visual perception of colouring is more even and healthier, with a higher proportion of light pink as compared to pink red, olive and beige. A similar and significant decrease in luminosity was observed in both groups and at both evaluation time points (-34.4%, p = 0.0001 and -33.3%, p = 0.0001 at W18). A slight decrease in brightness, which tended to be significant in the control-group 3 months after the last injection, was observed (-15.1%, p = 0.0856). A significant decrease in transparency was measured on the DRC-treated cheeks 3 months after the last injection (-16.3%, p = 0.0403).

Self-evaluation of DRC efficacy, by subjects

At both time points, the subjects, blinded to the treatment, were asked to evaluate the improvement of their skin status by using a 5 points scaling questionnaire. *Figure 2* shows results on the cheeks recorded 2 weeks after the last injection, when the treatment was expected to have optimum results. A trend for positive efficiency of the DRC-treatment was observed in the 4 parameters recorded, as the percentage of subjects rating as "improved" (or better than "improved") was higher for DRC treated side: for skin radiance 100% in the DRC side *versus* 92% in the control side, for skin quality 56% versus 52%, for skin comfort 48% *versus* 24% and for the lifting effect 56% *versus* 20%, respectively.

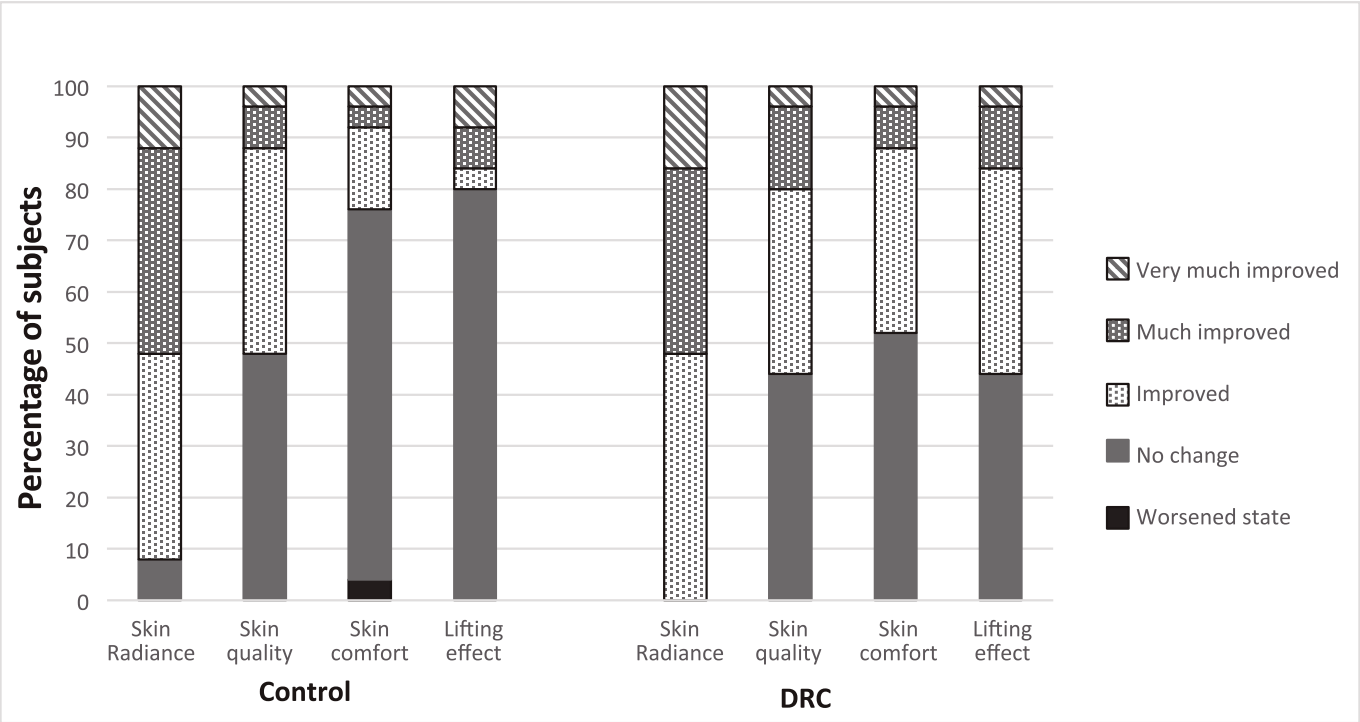

Figure 2. *DRC efficacy self-evaluation by subjects, compared with the control group at 2 weeks after the end of treatment using a 5-point scale (worsened state, no change, improved, much improved, very much improved).*

Score (mean ± SD)	D0	W8	% of change W8 *vs* D0	W18	% of change W18 *vs* D0
Pink red					
DRC	30.80 ± 9.09	22.00 ± 9.57	- 28.6***	21.20 ± 7.25	- 31.1‡
Control	29.20 ± 8.62	22.00 ± 9.12	- 24.6***	23.60 ± 7.57	- 19.2**
p value†	-	-	NS	-	0.0137
Olive					
DRC	41.60 ± 9.43	37.20 ± 6.78	- 10.6*	33.20 ± 6.27	- 20.2‡
Control	39.60 ± 10.98	35.20 ± 7.70	- 11.1*	34.40 ± 6.50	- 13.1*
p value†	-	-	NS	-	NS
Beige					
DRC	72.00 ± 10.40	56.40 ± 8.10	- 21.7‡	48.00 ± 9.12	- 33.3‡
Control	70.00 ± 11.54	54.00 ± 11.90	- 22.8‡	48.00 ± 9.57	- 31.4‡
p value†	-	-	NS	-	NS
Light pink					
DRC	66.80 ± 8.02	58.80 ± 11.66	- 12.0‡	60.00 ± 9.12	- 10.2***
Control	66.40 ± 9.52	55.60 ± 11.57	- 16.3‡	58.00 ± 7.07	- 12.6‡
p value†	-	-	NS	-	NS
Luminosity					
DRC	2.73 ± 0.90	2.22 ± 0.45	- 18.7***	1.79 ± 0.23	-34.4‡
Control	2.73 ± 0.92	2.28 ± 0.56	- 16.5**	1.82 ± 0.32	- 33.3‡
p value†	-	-	NS	-	NS
Brightness					
DRC	1.73 ± 0.70	1.69 ± 0.30	- 2.3	1.51 ± 0.29	- 12.7
Control	1.72 ± 0.72	1.71 ± 0.33	- 0.6	1.46 ± 0.32	- 15.1*
p value†	-	-	NS	-	NS
Transparency					
DRC	1.72 ± 0.61	1.76 ± 0.36	+ 2.3	1.44 ± 0.24	- 16.3**
Control	1.71 ± 0.62	1.76 ± 0.39	+ 2.9	1.46 ± 0.27	- 14.6*
p value†	-	-	NS	-	NS

* Comparison *versus* D0, slightly significant difference, $p < 0.1$, Wilcoxon test for Olive, and paired Student t-test for Brightness and for Transparency

** Comparison *versus* D0, $p < 0.05$, Wilcoxon test for Pink Red and Luminosity, and paired Student t-test for Transparency

Table 6. *Clinical scoring of skin, by a blinded trained assessor, on the cheek before (D0), 2 weeks (W8) and 3 months (W18) in each hemi-face after the end of treatment with DRC or the control in the ITT population using the C.L.B.T.™ sensory methodology.*

Safety and tolerance assessment

During the study all subjects reported at least one common local treatment reaction in their daily diaries (100% on the DRC-treated side vs 84% on the control side). 19% concerned both sides of the face, 70% only the side treated with DRC and 11% only the side treated with the control. Most of the reactions were mild to moderate in intensity with an average duration of 5 ± 3 days for the treated side and 4 ± 4 days for the control side. None of these local reactions led to any modification of the study protocol (all planned injections were administered). For the side treated with DRC, most reactions were swelling, bruising, erythema, firmness and irregularities. The most reported common treatment reactions concerned the neck (66% for the side treated with DRC). All common treatment reactions were resolved at the end of the study. All along the study, 57 adverse events were reported by the treating investigator in 17 subjects (such as rhinitis, headache, diarrhea, nausea, musculoskeletal pain, hypertension, flu, gingivitis).

No serious adverse event was reported. Among those, 98% of adverse events were deemed by the investigator as probably not related to the treatment and 2% (1 event) was deemed certainly not related to the treatment.

Generally, the treating investigator considered that the safety profile of DRC was very good and as expected for an HA injectable product for mesotherapy[2].

Discussion

This interventional, monocentric, randomized, double-blind, split-face comparative study showed a significant effect of DRC injections on skin roughness, and hydration on the cheeks in comparison with the control injections of a physiological saline solution. Skin elasticity and radiance were also improved at 3 months compared to the control side. The treatment with the DRC was shown to be appreciated by patients for its effects on the skin in terms of radiance, quality, comfort and lifting effect.

Indeed, repeated microinjections of DRC (3 injections, 3 weeks apart) led to a significant improvement of skin roughness at 8 weeks compared to the control side as revealed with the parameter *Sa*. At 3 months after the last injection, skin roughness returned to its baseline level (before treatment) but also worsened with the microinjections of physiological saline buffer. This result indicates that the treatment improves skin quality on the short term and that its effect lasts at least 3 months. The results on the secondary criteria also confirmed the improvement of dermis thickness, skin hydration, elasticity and radiance. Interestingly dermal thickening was observed, during the study, to reach a significant increase of 4.1% at 3 weeks after the last injection. The same result (+ 4%, p = 0.008) was observed by Baspeyras et al. at 3 weeks after 3 sessions of injection of a 14 mg/mL HA solution enriched with mannitol and glycerol (in both studies there was no statistically significant difference with the control), which was attributed to the induction by HA of dermis components such as elastin and collagen[2].

In the present trial, two weeks after the end of the treatment protocol, the DRC-treated side showed an increase in hydration of the dermis whereas hydration in the control group almost significantly decreased. Skin elasticity was also improved at 3 months in the DRC-group compared to the control group as revealed by the indentation value as well as the alpha and CoR parameters. Radiance of the skin also improved in the DRC group as observed with a higher proportion of light pink colour compared to pink red, olive and beige of the control group.

Thus, in the DRC-treated group, the skin appeared healthier and resulted in high patients' satisfaction with the quality of their skin condition. Although the mere dermal stimulation from the multipuncture procedure seems to have a positive short-term effect on the skin, these results demonstrate a significantly longer evolution on skin quality, as well as a return to the dynamics of young skin on the DRC treated side and would assure the effect of the study product and not only the procedure when compared to the control group.

The results suggest that the 14 oligonutrients may have a synergistic effect and/or may potentiate the effect of the high molecular weight HA (15 mg/mL) present in the DRC formula. Indeed, the 3 natural antioxidants may reinforce the effect of HA in the fight against the increased amount of free radicals in aged skin *(Table 7)*[1,12,13,14,15,16]. Leucine, isoleucine, valine, glycine, lysine, threonine, proline, vitamin B6, zinc and copper may support HA to restore the ECM by favouring the production of collagen but also by participating in other metabolic pathways that increase cellular metabolism. These molecules may then participate in tissue remodeling and also in cellular protection and regeneration. Finally, Arginine may increase the hydration of the tissue together with HA[14].

All these hypotheses would be proved further through *in vitro* and histopathologic studies. The physiological impact of DRC was previously examined on human skin explants[18]. In this *ex vivo* study, a bolus injection of DRC into the upper reticular dermis led, 9 days after the injection, to an increase of 26% in fibrillin as compared to the non-treated explants *(Table 8)*.

In addition a strong redensification of papillary dermis as well as the strengthening of the dermo-epidermal junction, associated with an increase of 98% of Collagen IV, were observed in this study[18]. The increase in the amount of collagen IV suggests the activation of fibroblasts and neosynthesis of the ECM components.

An increase of +1400% in glycosaminoglycans was observed in the epidermis suggesting that the hydration was also highly increased in this model. Finally, injections of DRC also decreased the oxidative stress since a reduction of 28% of the UV-induced malondialdehyde, a photo-induced oxidation marker, was observed.

Overall, these *ex vivo* tests demonstrated the efficacy of the microinjections of DRC formula with regard to the improvement of dermis structure as well as cellular protection against oxidative damage, cellular regeneration and tissue hydration. These observations of elastin and collagen overexpression after injection of DRC may contribute to the clinically observed benefits on elasticity and on the overall quality of the skin as shown in this trial after a protocol of 3 injections of the product 3 weeks apart.

Potential effects on dermis	Involved molecules	Mechanism of action
Antioxidant protection	High molecular weight HA	Scavenger of reactive oxygen species (ROS) [1, 13, 16]
	Glutathione	Main antioxidant of the cell, a powerful cellular protector [12]
	Alpha-lipoic acid	Universal endogenous antioxidant - Protects the cell and the cell's membranes; Recycles natural antioxidants and increases their duration [15]
	N-Acetyl-L-cystein	Antioxidant amino-acid - natural source of cysteine for the production of glutathione by the cell [14]
Tissue restructuring and cell regeneration	High molecular weight HA	Activation, differentiation, migration of fibroblasts and keratinocytes => Neosynthesis of collagen and HMW-HA that leads to the recreation of the ECM [1, 16]
	Glycine	One amino-acid of the glutathione tripeptide; Represents 1/3 of the triple-helix structure of collagen [19]
	Leucine, isoleucine, valine	Define the Branched-Chain Amino Acids group (BCAA) with known wound healing and tissues restructuring properties [20]
	Lysine, threonine, proline	All essential to the biosynthesis and to the stability of the triple-helix structure of collagen [9, 21, 24]
	Vitamin B6	Non-allergenic vitamin - Powerful antioxidant and co-factor of more than 140 biochemical reactions in cells; Essential for the cellular metabolism of all living organisms [22]
	Zinc and Copper	Minerals, essential constituents of natural antioxidant enzymes, they act in synergy with the other defense mechanisms of the cell [23]
Hydration	High molecular weight HA	Able to catch 1,000 times its weight in water – Deep dermis hydration [1]
	Arginine	Natural Moisturizing Factor (NMF); takes part in the hydration regulation of the superficial layers of the skin [17]

Table 7. *Potential synergistic effects of DRC oligonutrients on skin quality and health.*

Potential effect of the dermis	Marker
Antioxidant protection	-28 % malondialdehyde
Tissue restructuring and cell regeneration	+ 26% fibrillin + 98% collagen Papillary dermis densification
Hydration	+ 1400% glycosaminoglycans

Table 8. *Beneficial effects of a bolus injection of DRC into the upper reticular dermis of skin explants versus non treated explants after 9 days.*

Conclusion

Our study, by a series of biometrologic measurements, confirmed the theoretical benefits of high molecular weight HA microinjections on skin health and also suggests that the addition of selected oligonutrients may increase the efficacy of this molecule on skin biorejuvenation. The split-face design of this randomized, double-blind, comparative study clearly demonstrated the benefits of repeated injections of DRC. Altogether, these data suggest that this treatment can prevent premature ageing as well as the appearance of small wrinkles due to ageing and/or UV irradiation.

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aesthetic medicine

Review

Emerging alternative topical therapies in the treatment of solar lentigo and post-inflammatory hyperpigmentation: a review

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Abstract

Acquired cutaneous hyperpigmentation disorders, namely melasma, post-inflammatory hyperpigmentation and solar lentigo, are a common and challenging complaint in the field of esthetic medicine. They substantially contribute to the aged appearance of skin and impact a patient's quality of life. While numerous topical agents - particularly hydroquinone, kojic acid and retinoids - have been extensively studied and provide remarkable improvement of the problem, their limited skin tolerability continues to contribute to lowered efficacy and a lowered rate of compliance. This review looks into the newer, safer and more natural alternative pigment-regulating agents such as niacinamide and N-acetyl glucosamine and the potential of integrating them into the already well-established skin pigment control protocols in order to obtain better results.

Keywords

Hyperpigmentation, hydroquinone-free, niacinamide, N-acetyl glucosamine

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Introduction

Cutaneous pigmentation disorders are caused by alterations in the production of melanin in the skin. Causes are varied and include exposure to ultraviolet (UV) radiation, inflammatory conditions, hormonal imbalances and drugs. Although there is limited literature addressing the exact prevalence of such disorders, dyspigmentation is a common complaint, increasingly encountered in daily practice. It is particularly concerning in patients with darker skin types (Fitzpatrick IV-VI)[1]. The general aesthetic desire and primary focus of many cosmetic-based industries is uniformity of skin colour. While being notoriously difficult to treat, many cosmetic-pharmaceutical hybrids have emerged and the race is now on to find safer and more efficacious skin lightening agents.

Physiological regulation of melanogenesis and melanosome transfer

Cutaneous pigmentation is a result of the blending of: oxyhemoglobin (red) and deoxyhemoglobin (blue) in blood vessels, beta-carotene (yellow-orange) in blood, and the biosynthesis (melanogenesis) of two major natural black/brown/red pigments - eumelanin and pheomelanin[2]. In human skin, melanins serve as broadband UV absorbents with antioxidant and radical scavenging properties and hence protect nuclear DNA from mutations caused by ionizing UV radiation[3]. Melanogenesis of both these pigments occurs in highly specialized cytoplasmic organelles of dendritic melanocytes, known as melanosomes. The melanocytes, together with keratinocytes, reside along the basement membrane in the stratum basale of the epidermis of the skin[4], immediately up to the dermo-epidermal junction. Together, they form the epidermal melanin unit - consisting of a melanocyte and its direct interaction with an associated population of approximately 36 keratinocytes[5]. The epidermal melanin unit plays an important role in a chain of complex biochemical events, resulting in the production and transfer of melanin pigments. While the intricate processes of melanin production and transfer are beyond the scope of this text, a basic overview of the series of enzymatic and spontaneous reactions involved in melanogenesis are outlined below. The site of melanogenesis and melanin pigment storage is the melanosome within the melanocyte[6]. Among numerous intrinsic and extrinsic factors which regulate the pigmentary response, melanocortin peptides such as α-melanocyte stimulating hormone (αMSH) and adrenocorticotropic hormone (ACTH) are the key mediators, exerting their action via the seven-transmembrane G-protein-coupled melanocortin-1 receptor (MC1-R) found on melanocytes[7]. Both of these melanocortin peptides arise from the pars intermedia of the pituitary gland, by the proteolytic cleavage of the larger precursor molecule pro-opiomelanocortin (POMC) following UV exposure. It is interesting to note that due to the unsubstantially developed pars intermedia in adults, epidermal keratinocytes and melanocytes are the second major source of melanocortin peptides[8,9]. αMSH and ACTH are agonists at MC1-R, leading to the conversion of cytoplasmic ATP to cAMP. Increased levels of cAMP further activate protein kinase A (PKA) which translocates to the nucleus and induces expression of microphthalmia-associated transcription factor (MITF), ultimately promoting transcription of melanogenic enzymes and melanogenesis.

Both eumelanin and pheomelanin are subsequently synthesized from the amino acid precursor L-tyrosine through a series of reactions catalyzed and coordinated by the aforementioned melanogenic enzymes, namely tyrosinase (TYR), DOPAchrome tautomerase (DCT) and tyrosinase-related protein-1 (TYRP1) – the pathway being frequently referred to as the Raper-Mason pathway[11].

Once formed, the pigment-loaded melanosomes migrate towards the melanocyte dendritic tips and are transferred to the neighbouring keratinocytes[12], forming protective melanin caps over the nuclei[13]. The precise molecular and cellular mechanisms of this transfer have still not been clearly defined, however, several possible transfer mechanisms have been proposed. Seiberg's research on keratinocyte-melanocyte interactions suggests the transfer occurs via melanosome ingestion and receptor mediated phagocytosis by keratinocytes. A seven-transmembrane G-protein PAR-2 was found to mediate the ability of keratinocytes to phagocytose melanosomes. In-vitro studies showed that PAR-2 activation induced a dose-dependent, statistically significant increase in phagocytosis of melanosomes by keratinocytes while PAR-2 inhibition by soybean trypsin inhibitor (STI) resulted in the opposite effect[12].

Ando, Niki, Yoshida et al. proposed a novel mechanism for the transfer of melanosomes where pigment globules (melanophagolysosomes) containing multiple melanosomes are released from melanocyte dendrite tips into the extracellular space and are subsequently ingested by keratinocytes[14].

Scott, Leopardi, Printup and Madden investigated melanosome transfer through time-lapse digital imaging of melanocyte-keratinocyte co-cultures. They detected melanosomes within dynamically active melanocyte filopodia (cytoplasmic projections containing actin filaments) which attached to keratinocyte membranes suggesting direct inoculation of melanosomes into keratinocytes[15]. It is highly probable that the transfer of melanosomes to keratinocytes occurs by multiple simultaneous mechanisms, and future studies are likely to uncover precise transfer mechanisms which will result in better control of human pigmentation.

Pathophysiology of acquired hyperpigmentation

Pigmentary disorders can be classified into two distinct groups: disorders of quantitative/qualitative distribution of normal pigment and disorders with abnormal presence of exogenous/endogenous pigments in skin[2]. Acquired hyperpigmentations fall under the first group and can further be subclassified into:

- epidermal hypermelanocytosis/hypermelanosis - increased number of melanocytes/increased melanin production in basal and suprabasal layer
- dermal hypermelanocytosis/hypermelanosis (ceruloderma) - ectopic dermal melanocytes/ pigmentary incontinence where melanin leaks into the

extracellular matrix of the dermis or accumulated in dermal melanophages
mixed hypermelanosis - co-involvement of both
· epidermal and dermal hypermelanocytosis/ hypermelanosis[16].

Keeping in mind the physiologic melanogenesis process, we turn to the pathophysiology of acquired hyperpigmentations which this review will address, namely post-inflammatory hyperpigmentation (PIH) and solar lentigo. PIH represents the sequelae of the epidermal response following inflammation of the skin either by mechanical trauma, cutaneous diseases (dermatitis, acne, allergic eruptions etc.) or therapeutic/cosmetic procedures (lasers, microdermabrasion, radiofrequency etc.).

It is presently postulated that immune-inflammatory cytokines and arachidonic acid metabolites are mainly responsible for the increase in melanocyte activity during skin inflammation processes[17].

The membrane-derived inflammatory compounds leukotriene C4 (LTC4) and leukotriene D4 (LTD4), and to a lesser extent prostaglandin D2 (PGD2), thromboxane-2, interleukin (IL)-1 and IL-6 were shown to stimulate melanocyte proliferation and dendricity as well as cause an increase in the amount of tyrosinase[18,19,20].

Following UV exposure, another well-known ubiquitous inflammatory factor - histamine - is increasingly produced and released from dermal mast cells[21]. Recently Yoshida et al. proved histamine involvement in pigmentation (increases in tyrosinase activity) via H2 receptors on cultured melanocytes[22].

The increased production of melanin, as well as its abnormal distribution throughout the papillary dermis and epidermis[23] explains why epidermal hypermelanosis/hypermelanocytosis occurs following many inflammatory states of the skin.

Solar lentigo is associated with photo-ageing, resulting from chronic ultra-violet (UV) radiation exposure leading to irregular pigmentation.

A lentigo appears as a pigmented macule, usually sharply demarcated, surrounded by normal-appearing skin. Histologic features include epidermal hyperplasia and increased pigment in the basal layer.

Solar lentigines may serve as clinical markers for previous sunburn and could point to higher risk of developing cutaneous melanoma[24].

Following sun exposure, immediate increase in pigmentation occurs from the oxidation of pre-existing melanin and redistribution of pre-existing melanosomes[25].

Delayed increases in pigmentation occur several days after UV exposure and involve keratinocyte and fibroblast production and secretion of several paracrine factors which promote melanocyte function, survival, proliferation and dendticity.

These include endothelin-1 (ET-1), nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and α-melanocyte stimulating hormone (αMSH). UV photons also directly stimulate the phosphorylation of melanocyte cytoplasmic tyrosinase leading to increased melanin production and deposition[26].

Simultaneously UV-induced DNA damage results in DNA photoproducts (mostly thymine dimers) which have been shown to increase melanogenesis and double melanin concentration in human melanocytes[27].

These DNA photoproducts increases synthesis of the rate-limiting enzyme of melanogenesis, tyrosinase[28], and increases cell surface binding of α-melanocyte stimulating hormone (aMSH)[29].

Andersen et al. investigated histopathology of 51 solar lentigo biopsies stained with hematoxylin-eosin, Mel-5 immunoperoxidase and Fontana-Masson stains to quantify melanocytes and melanin content in the skin. They discovered a 2.2-fold increase in melanin content which was evenly dispersed in the basal layer and/or scattered in melanophages in the papillary dermis[30].

Diagnosis

Both PIH and solar lentigo typically present as macules or darkened patches over initial inflamed or sun-exposed areas and commonly cause significant psychosocial distress to the patient. The pigment location may be determined by a Wood's lamp examination. Since 1903, the Wood's light has been a useful tool for evaluating cutaneous pigment disorders and skin infections. The Wood's lamp radiation comes from a high-pressure mercury arc lamp, filtered by a barium silicate and 9% nickel oxide filter[31] and emits a light of a wavelength between 320-400nm, peak at 365nm (UV range)[32]. Human skin contains numerous fluorescent compounds, including collagen, elastin and co-enzymes which reflect UV light and produce bright blue-white patches under Wood's lamp investigation[33].

Melanin on the other hand, has a natural tendency to absorb UV radiation and therefore diminishes the intensity of the Wood's light, making areas containing more melanin appear darker than the rest of the normal skin. Furthermore, the Wood's lamp has the ability to additionally accentuate (darken) superficial melanin, since insufficient quantity of UV radiation can penetrate into the dermis, allowing for differentiation between epidermal and dermal melanin deposits[34]. Localization of pigment location in acquired hyperpigmentation is of crucial importance when formulating the appropriate management and treatment plan. Superficially located epidermal melanin is more likely to respond to topical treatments than dermal melanin deposits.

Targets for topical pigmentation control

As depicted above, the processes involved in abnormal skin pigmentation are profoundly complex. Establishing targets for effective and permanent inhibition continues to intrigue researchers worldwide, with a particular interest from the cosmeceutical industry. To date, a wide array of pigmentation control agents have successfully been identified.

Hydroquinone (1,4-dihydroxybenzene)

The most common target for pigmentation control is the rate-limiting enzyme tyrosinase, which catalyzes the oxidation of tyrosine into dopaquinone. This step is the only rate-limiting reaction in melanogenesis since subsequent reactions all proceed spontaneously at

physiological pH values[35]. As tyrosinase is only present in melanocytes, agents inhibiting this enzyme are highly specific to solely inhibiting melanogenesis. The most notorious and potent tyrosine inhibitor, in vitro and in vivo, is hydroquinone (HQ) – a phenolic compound which primarily inhibits the enzymatic oxidation of tyrosine and phenol oxidases[36]. It may also inhibit RNA and DNA synthesis thereby altering and/or damaging melanocyte function[37]. As demonstrated in multiple studies over the years, these two inhibitory processes significantly suppress melanin pigment production and gradually reduce acquired hyperpigmentation[38,39,40,41].

Despite its powerful depigmentation ability, HQ has lost popularity recently due to the increased reported incidence of adverse effects, ranging from erythema and irritation of the skin to disfiguring ochronosis (excessive skin darkening)[42,43] or halo depigmentation (hypopigmentation of skin surrounding treated area). The pathogenesis of ochronosis remains unclear to date. Speculation about this excessive formation of pigment currently focuses on HQ penetrating into the papillary dermis thereby causing phenolic degradation products[44] and local inhibition of homogentisic oxidase by HQ which causes accumulation of homogentisic acid which polymerizes into ochronotic pigment in the papillary dermis[45,46]. Excessive and at times permanent halo depigmentation occurs as a result of various melanocytotoxic HQ metabolites which are oxidized within the treated melanocytes[47].

In 1982, the Food and Drug Administration (FDA) reviewed available data and published a rule stating that skin bleaching drug products containing 1.5%-2% HQ are generally recognized as safe and effective (GRASE)[48]. In 2006 however, the FDA proposed to withdraw the 1982 rule, as evidence of HQ acting as a carcinogen and cases of ochronosis emerged. Therefore, the new FDA recommendation in 2006 regarding HQ usage stated that additional studies need to be conducted in order to determine whether HQ poses any risk to humans. They further confirm that HQ should remain available as an over-the-counter (OTC) drug product pending final ruling[49]. The current commonly used OTC concentrations of HQ are up to 2%, but strengths up to 10% may be prescribed in the United States[36]. In the European Union, HQ is entirely banned in cosmetic products ("any substance or mixture intended to be placed in contact with the external parts of the human body")[50].

Attempts to improve HQ potency and to reduce adverse effects have been done by combining HQ with supplemental pigment-lightening agents such as glycolic acid, tretinoin, and antioxidants, sunscreen ingredients and corticosteroids – all of which enhance HQ penetration, deliver HQ in controlled doses and further inhibit melanogenic processes. Cook-Bolden investigated usage of a combination cream (Glyquin) in patients with PIH which contained HQ 4%, buffered glycolic acid 10%, vitamin C, vitamin E and sunscreen for 12 weeks. Based on spectrophotometric readings of lesions before and after treatment, the degree of pigmentation in treated areas decreased significantly when compared to untreated areas. He further investigated an innovative product containing microencapsulated HQ 4% with retinol 0.15% and antioxidants and concluded similarly successful results[51,52].

Mequinol (4-hydroxyanisole)

Mequinol is the prescription alternative to HQ and is readily available in both the US and EU member states. Similarly to HQ, mequinol mechanism of action is competitive inhibition of tyrosinase and subsequent inhibition of melanogenesis[53]. The main reason why mequinol is preferred over HQ is the fact that 4-hydroxyanisole is proven to cause no specific toxic effect and no morphological damage to melanocytes at the ultrastructural level[54]. Multiple large clinical studies over the years have proven the efficacy of mequinol as adequate alternative to HQ in acquired epidermal hyperpigmentation[55,56,57].

Arbutin (hydroquinone-O-β-D-glucopyranoside)

A natural substance known as arbutin, and its more potent synthetically produced derivative deoxyarbutin, have emerged as botanicals similar in structure and function to HQ. Obtained from leaves of bearberry, cranberry and blueberry plants, arbutin and deoxyarbutin inhibit tyrosinase competitively and reversibly but unlike HQ, have significantly less melanocytotoxic potential and minimal influence on melanocyte tyrosinase mRNA expression[58,59,60]. A series of studies conducted on arbutin and deoxyarbutin pigment-lightening abilities in solar lentigo and PIH concluded that these HQ glucoside derivatives exert potent tyrosinase inhibition and may serve as safe alternatives to HQ[61].

Kojic and Azelaic acids

Moving away from quinone-related agents, we turn to Kojic and Azelaic acids - both substances originating from microorganisms Acetobacter/Aspergillus/Penicillium and Pityrosporum ovale respectively[62]. They act as tyrosinase competitive inhibitors - Kojic acid chelates copper[63] while Azelaic acid binds to amino and carboxyl groups at tyrosinase active site[64] - preventing melanogenesis in a similar fashion to the above mentioned quinone-related agents with highly effective results in both treatment of solar lentigo as well as PIH[64,65,66]. Kojic acid is available in 1% - 4% formulations and usually in combination with other pigment regulating compounds. Considered to have a high sensitizing potential, the biggest concern is the high frequency of contact dermatitis associated with its use[67,68]. Azelaic acid preparations usually contain 20% acid and have not shown any alarming side effects when used appropriately in the treatment of epidermal hyperpigmentary disorders.

Tranexamic acid

Tranexamic acid (TA, trans-4-aminomethyl cyclohexane carboxylic acid) is essentially a plasmin inhibitor. It is commonly used to prevent and treat excessive blood loss from trauma or surgery and other medical conditions including hemophilia[69]. Oral, intradermal and 2-3% topical preparations of TA have recently demonstrated ameliorable effects on hyperpigmentary disorders. The mechanism appears to be of an anti-inflammatory nature, however, the exact effect on melanogenesis and related pathways has not yet been elucidated. Some studies are based around the fact that plasmin promotes the production and release of

arachidonic acid and the synthesis of prostaglandins from UV irradiated keratinocytes, therefore TA acts to prevent the inflammatory response which ultimately results in the inhibition of prostaglandin-induced melanogenesis[70,71]. Results from another study suggest that TA may lead to degradation of MITF which then downregulates transcription of tyrosinase mRNA and hence tyrosinase activity[72].

Exfoliating agents

A different approach to skin-lightening is the removal of keratinocytes containing melanin in the uppermost layer of the epidermis. This may be achieved with agents inducing exfoliation, commonly α-hydroxyacids such as glycolic acid, or agents that increase epidermal turnover rate, such as retinoids. Glycolic acid is the smallest α-hydroxyacid as well as the most versatile. Owing to its low molecular weight, it has good penetration and bioavailability and is easily neutralized and controlled[73]. It has an epidermal remodeling effect and at concentrations of 20% - 70% can be used in the treatment of epidermal hypermelanoses and is well tolerated even in Fitzpatrick skin types IV-VI[74]. Topical retinoids ie. all-trans-retinoic acid (RA), 13-cis-retinoic acid (isotretinoin), retinol and retinaldehyde, all improve the appearance of epidermal hyperpigmentation by modulating epidermal cell proliferation, differentiation and cohesiveness[75,76].

Retinoid concentrations range from 0.01% - 0.1% and titration should be done progressively based on treatment response so as to avoid the well known retinoid dermatitis which may exacerbate epidermal hypermelanoses[77].

Emerging agents for pigment control

The broader understanding of the potential hundreds of genomic and proteomic processes involved in melanogenesis, and an ever-growing consumer need, the search for safer and more tolerable and effective pigment control agents continues. This brings us to a few of the newer concepts in pigment lightening therapy. We begin with niacinamide (nicotinamide) and active metabolite of vitamin B3. Although niacinamide does not show significant inhibition of tyrosinase nor does it have influence on the number of melanocytes, it hinders melanosome transfer from melanocytes to keratinocytes - another crucial target in the inhibition of the melanogenic process[78]. The proposed mechanism for this inhibition of melanosome transfer is niacinamide's ability to modulate protease-activated receptor (PAR-2) - a seven-transmembare G-protein-coupled receptor found on keratinocytes but not on melanocytes. PAR-2 regulates keratinocyte phagocytosis of melanosomes and its inhibition leads to significant reduction of pigment transfer, abnormal melanosome dynamics and processing with subsequent depigmentation. Seiberg et al. hypothesize that the inability of keratinocytes to receive melanosomes causes accumulation of melanosomes in melanocytes dendritic processes which initiate a negative feedback mechanism and reduce melanin pigment production[12,79]. Niacinamide has good skin penetration and bioavailability showing maximum absorption at 48-72 hours[80] while return to normal melanosome transfer function occurred 72 hours after removal of niacinamide[81]. Formulations of 2-4% niacinamide have shown good to outstanding results in cases of epidermal hypermelanoses, with no significant side effects and good tolerability - suggesting its potential for long-term usage as part of hyperpigmentation maintanence therapy[82,83,84,85,86].

The second novel agent is an amino sugar N-acetyl glucosamine (NAG), found in all human tissues and recently proven to reduce the production of melanin in melanocytes in vitro and in vivo[87,88]. NAG primarily inhibits the glycosylation of pro-tyrosinase to the active tyrosinase thus reducing tyrosinase activity and melanogenesis[89].

Kimball et al.[87] conducted a randomized, double-blind, vehicle-controlled investigation on 202 caucasian women (40-60 years of age) with moderate-moderately severe irregular hyperpigmentation primarily due to solar lentigines. Half of the participants applied a morning and night moisturizer containing 4% niacinamide + 2% NAG with sufficient sun protection factor (SPF) agents while the second group used a non-active vehicle formula with the same SPF agents. Subjects were asked to apply the moisturizers twice daily (a total of approx. 2g) for 8 weeks, with clinical evaluation done at baseline, weeks 4, 6 and 8 with facial colour imaging and noncontact SIAscopy™, described in previous literature[90,91]. Final results of the study showed the 4% niacinamide + 2% NAG preparation was consistently more effective than the vehicle in reducing appearance of hyperpigmentation. Most importantly, it was well tolerated and resulted in no adverse effects[87].

Conclusion and Discussion

Given the complexity of cellular and biochemical pigment production processes, multiple agents are able to regulate and inhibit various steps of melanogenesis *(Figure 1)*[10]. Despite the lack of evidence of any malignancies associated with topical HQ use - the formerly accepted 'gold-standard' of skin lightening has been challenged and banned due to reported grave side effects[49]. Research focus has shifted onto novel agents and their potential to safely and effectively control pigmentation. These agents may mimic HQ tyrosinase inhibition, as mequinol and arbutin do, or they may interfere with the interaction between keratinocytes and melanocytes during melanosome transfer, as demonstrated by niacinamide. Other potential agents, which go beyond the scope of this review, include antioxidants[92] microphthalmia-associated transcription factor (MITF) inhibitors[93] and sex hormone regulators[94].

No matter the mechanism of action targeted, it is clear that there is a dire need for standardized strategies and protocols for acquired cutaneous hyperpigmentation disorders. Discussed above are the details of some of these alternative agents - their individual formulation, toxicology, skin tolerability and efficacy. The concept of targeting more than one step in the pigmentation process simultaneously should also be stressed - the synergistic effect on skin lightening by multiple agents has been proposed and verified by numerous published studies: Fleischer et al.[53] tested 1,175 subjects with

solar lentigenes using combined 2% mequinol and 0.01% tretinoin and proved clear superiority over results obtained with each of the agents individually while Kimball et al.[87] investigated 202 subjects using a combined formulation of 4% niacinamide and 2% NAG concluding superior effectiveness with combined use. The recommendation of a multi-agent approach to epidermal melanoses calls for further broad-scale investigation and follow-up.

Finally, an invaluable agent in the treatment of undesired cutaneous hyperpigmentation is a broad-spectrum sunscreen with a sun protection factor (SPF) of at least 30. Proper patient education and counseling on daily sun-protective measures cannot be stressed enough.

Limitations which exist in most studies conducted and cited here include: a small sample size, lack of double-blind and placebo trials, lack of standardized pigment assessment pre and post treatment and lack of histopathological assessment. These should all be considered in further investigations in order to achieve more successful outcomes in the future.

In conclusion, significant evidence points to the fact that acquired cutaneous hyperpigmentations, especially epidermal hypermelanoses, require long-term topical treatment and respond best to combination therapy which targets multiple steps of melanogenesis simultaneously. Further investigations, both in vitro and in vivo, are necessary to expand on these recent findings and to help establish a much-needed new 'gold-standard' protocol to battle the present problem of acquired cutaneous hyperpigmentation.

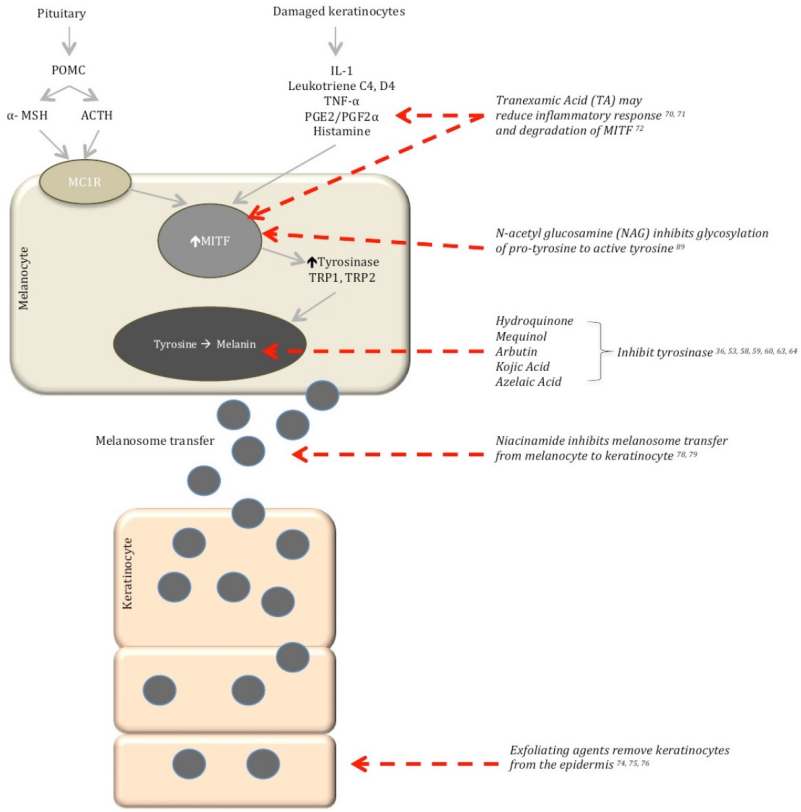

***Figure 1**. Melanogenesis and agents that may regulate it.*
POMC=Pro-opiomelanocortin, a-MSH=Alpha-Melanocyte Stimulating Hormone, ACTH=Adrenocorticotropic Hormone, MC1R=Melanocortin-1 receptor, IL-1=Interleukin-1, TNF-a=Tumor Necrosis Factor-alpha, PGE2=Prostaglandin E2, PGF2a=Prostaglandin F2-alpha, MITF=Mocrophthalmia-associated transcription factor, TRP=Tyrosinase-related protein.

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aesthetic medicine

Original Article

Treatment of Androgenic Alopecia with Autologous Skin Micrografts

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Abstract

The possibility of treating or improving certain conditions using biological materials developed from the patient's own tissues has always been a very interesting idea. Skin is a very appealing tissue for regenerative aesthetic medicine. In the context of aesthetic medicine, a series of treatments have successfully opened the way: procedures with mechanical disintegration of donor tissue. These treatments are particularly interesting because no chemicals, enzymes or complex processing are used. The Rigenera® method uses a special micro-dermatome that breaks up the structure of skin obtained by means of biopsies and filters elements smaller than 50 microns[4], such as cells. The processed material is re-injected into the patient's skin, at the site of the condition to be treated. The purpose of this work is to assess the results obtained during one year of treatment in patients with alopecia using a skin micrograft technique with the Rigenera® system.

Keywords

Stem Cells, re-cell, adipose-derived stem cells, micrografts, Rigenera

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Introduction

Life expectancy of humans has increased significantly in recent years. This has underlined the issue of the deleterious effects of ageing, due to the high incidence of age-related conditions. The possibility of curing or improving certain conditions using biological materials developed from the patient's own tissues has always been a very interesting idea. Regenerative medicine is now a reality[1], with treatments applied to an increasing number of patients and for various purposes.

Skin is a very appealing tissue for regenerative aesthetic medicine. There are several reasons for this. First, it can be accessed easily and immediately[2]: all materials necessary to start certain procedures can be obtained by means of a conventional biopsy. Second, most aesthetic treatments are applied on the skin itself. The fact that donor biological material comes from the same tissue where it will be later injected is not trivial, since it lowers the legal classification of the procedure and makes application easier. However, if skin was processed and injected into different tissues some of these treatments would be classified as "cell treatments" or "advanced therapies" and as such would be subject to much stricter and more restrictive legislation.

It was first believed that the future of regenerative medicine applied to aesthetic medicine would be based on cell cultures. The possibility to select a certain type of cell, such as fibroblasts, and culture them for later use at a target site was of course very interesting. The possibility to expand the clone or increase the number of cells, also the possibility of using their derived products were promising. The cells could be made to synthesize certain proteins (such as collagen) and those proteins, rather than the entire cell, would be re-injected. However, isolating certain cells was not all that easy and neither was culturing them.

Practically the same thing happened with stem cells (SCs). At first scientists and then doctors stumbled upon the problems of working with SCs. Controlling cell division and directing cell differentiation toward a certain lineage proved to be a titanic task along with dedifferentiation, which involved retracing the steps of the cell back to the crossroads where it was forced to pick a development path toward a different cell lineage. This sounded like science fiction. In 2012, Yamanaka and Gurdon[3] showed that all this was possible by reprogramming adult cells into SCs. For the world of high-complexity and high-budget medicine, SCs became a reality with enormous potential and some treatment usefulness. Difficulties were mainly legal, economic, ethical, and regulatory. However, the circumstances of SCs related to aesthetic medicine are quite different.

In the context of aesthetic medicine, a series of treatments have successfully opened the way i.e. procedures with mechanical disintegration of donor tissue. These treatments are particularly interesting because no chemicals, enzymes or complex processing are used. Devices are available which work by breaking up tissue structure and concentrating, but not isolating, certain cells. It is worth noting that manufacturers are beginning to understand the specific needs of regenerative medicine protocols applied to aesthetic medicine. These new devices are very easy to use, virtually automate all parameters and steps in the process and, most importantly, are self-contained. They prevent graft contamination at any step of the process and eliminate the need for a doctor to process the graft. This means that they are not operator-dependent. This ensures the quality of the product injected, regardless of who processes the tissue, which in turn makes it easier to standardize protocols and compare results.

The Rigenera® method uses a special micro dermatome that breaks up the structure of skin obtained by means of 2.5 mm biopsies and filters elements smaller than 50 microns[4], such as cells. The processed material is re-injected into the patient's skin, at the site of the condition to be treated. Fiber proteins and the stratum corneum are naturally excluded, since the cell fraction obtained is the biological product needed with therapeutic action. No added chemicals or any other physical processing medium is used. This method is not only used in the treatment of androgenic alopecia, it has been used for several years in the treatment of chronic wounds and ulcers[5] of various etiologies, in the management of acute wounds and subsequent scars[6], in the treatment of joint conditions and associated pain, in bone regeneration[7] and in dental conditions[8].

The purpose of this work is to assess the results obtained during one year of treatment in patients with alopecia using a skin micrograft technique with the Rigenera® system.

Materials and Methods

Subjects were recruited consecutively between June 1, 2015, and December 15, 2016, among people who visited Mediestetic in Madrid, Valdemoro and Toledo and who satisfied the following inclusion criteria: i) different stages of alopecia as motive for consultation, ii) tests including PSA and hormone profile, iii) androgenic alopecia diagnosis, iv) no concomitant conditions of the scalp, and v) no systemic conditions. The treatment protocol was the following:

- Preparation. Hair washed and no hairspray. Application of topical ozone in a bag for 15 minutes.
- Selection of the donor area. Area of the scalp not sensitive to the action of testosterone where greater follicle density is identified. Samples extracted from the occipital region.
- Hair in the extraction area marked and cut.
- Preparation of materials to be used in the intervention: surfaces, antiseptic, anaesthetic, Rigeneracons®, and other perishables.
- Peripheral local anaesthetic. Lidocaine 2% without adrenaline in a bleb.
- Micrograft obtained. 4 mm punch.
- Micrograft washed with saline.
- De-epithelization and fragmentation with 11 blade.
- Micrograft placed in the Rigeneracons® device, on the rack. 1.5 ml of saline at room temperature added.
- The Rigeneracons® device is inserted into the machine which performs mechanical disintegration at 80 rpm: One or two 1-minute or 2-minute cycles until no traces of solid biological material can be seen inside.
- Recovery of material obtained with a 2 ml syringe.
- Deep intradermal injection in the receiving area with 29G x 12 mm needles.

- The entire process is repeated with a new ml of saline to obtain a second, lower-concentration cell suspension, which is then injected in the same way.
- Home care instructions, appointment for follow-up one month later and removal of stitches from the donor area.

The degree of alopecia was rated on the Ludwig scale for women and on the Hamilton-Norwood scale for men, including the A (front) and V (vertex-crown) variants for types III to V-[9].

All patients were prescribed supplemental Minoxidil 2% associated with Finasteride 0.15%[10]. In the case of patients who were already undergoing an established treatment, this was maintained unchanged.

Patient satisfaction was rated on a 7-point scale from -3 to +3: +3 for intense hair growth, +2 for moderate hair growth, +1 for mild hair growth, 0 for no change, -1 for mild hair loss, -2 for moderate hair loss, and -3 for intense hair loss.

A simple scale with only three categories (improvement, no change, and worsening) was developed for subjective results assessment by the physician.

The Rigeneracons® device is a sterile Class I CE medical device manufactured by Human Brain Wave srl in Italy. Technical specifications are as per insert.

The Rigenera® machine is a Class I CE medical device manufactured by Human Brain Wave srl in Italy. Technical specifications are as per insert.

Results

The sample included 44 subjects (28 men and 16 women), with a mean age of 48.05 years (SD 11.77).

The severity of alopecia on the Hamilton-Norwood scale (from 1 to 7, for men) was a mean of 4.36 (SD 2.33) and a median of 4. The severity of alopecia on the Ludwig scale (from 1 to 3, for women) was a mean of 2.38 (SD 0.62) and a median of 2. Using both scales jointly, the severity of the cases of androgenic alopecia treated was as follows *(Figure 1)*: 18 severe cases (40.91%), 24 moderate cases (54.54%), and 2 mild cases (4.55%).

Assessment of final results by the physician was +2 (median), in a discrete scale (from -3 to +3). The mean was +1.83 and dispersion was 0.88 (SD).

Assessment of final results by the patient *(Figure 2)* was: “very good” in 20.46% of cases; “good” in 59.09% of cases; “fair” in 11.36% of cases; and “no change” in 9.09% of cases.

Tolerance to the procedure was considered “very good” by patients in 100% of cases.

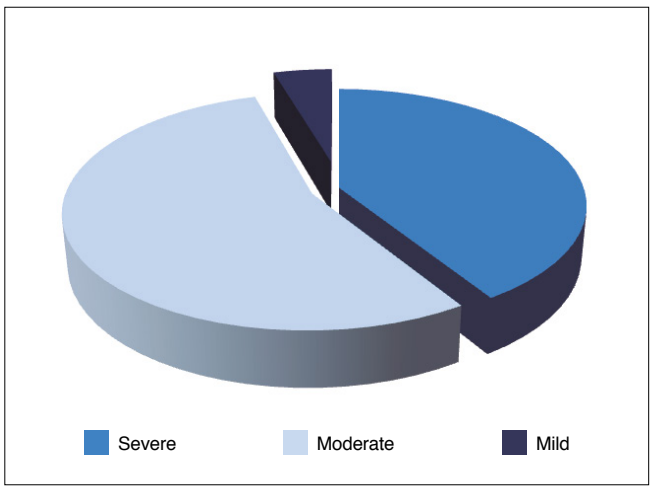

***Figure 1**. Severity of the cases of androgenic alopecia treated.*

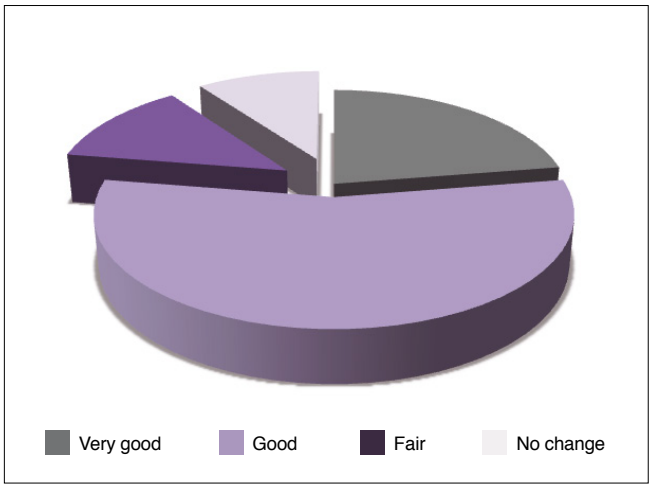

***Figure 2**. Assessment of final results by the patients.*

Discussion

The results we have obtained are very good in general *(Figure 3)*.

From the point of view of the treating physician, the improvement observed in patients has been very significant. On the other hand, the patients themselves confirmed the improvement observed by the physician, since 79.55% of patients rated treatment results as "good" or "very good", while only 2 patients did not perceive any results. In addition, all patients reported high ("very good") tolerability and many of them noticed slight darkening of the hair, which was deemed a very positive development.

However, there are certain issues that need addressing for an appropriate development of future research with this product. Firstly, physicians should manage patient expectations adequately. It must be made clear before intervention that expected results are not comparable to the results of FUE or FUSS hair transplant surgery. In fact, joint application of these techniques is reasonable and probably very useful. Future studies should test this hypothesis. Secondly, this work is not valid for an appropriate assessment of the technique's efficacy, since many of the patients were undergoing other related treatments. Our purpose has been to explore solutions to satisfy the demands of our patients. Another type of study, specifically designed with different aims and methodologies to isolate this variable, is required.

Although the number of patients in this observational study is small (44), after a brief period of time observing our patients we can state the following:

1. No significant adverse effects have been observed.
2. Tolerance has been very good.
3. The treatment is very safe.
4. The results obtained are satisfactory for most patients.

Lastly, it is important to understand how this type of treatment is performed within the current scenario of aesthetic medicine. Virtually all aesthetic medicine treatments are administered to out-patients, unlike many regenerative medicine treatments, which require sterile conditions and certain infrastructures. Aesthetic physicians should be trained to work under these conditions and to comply with the specific needs and standards of regenerative medicine treatments applied to aesthetic medicine. This is a blooming and fast-growing area, but at the same time it requires reliable, effective, and simple solutions.

Conflicts of interest

None.

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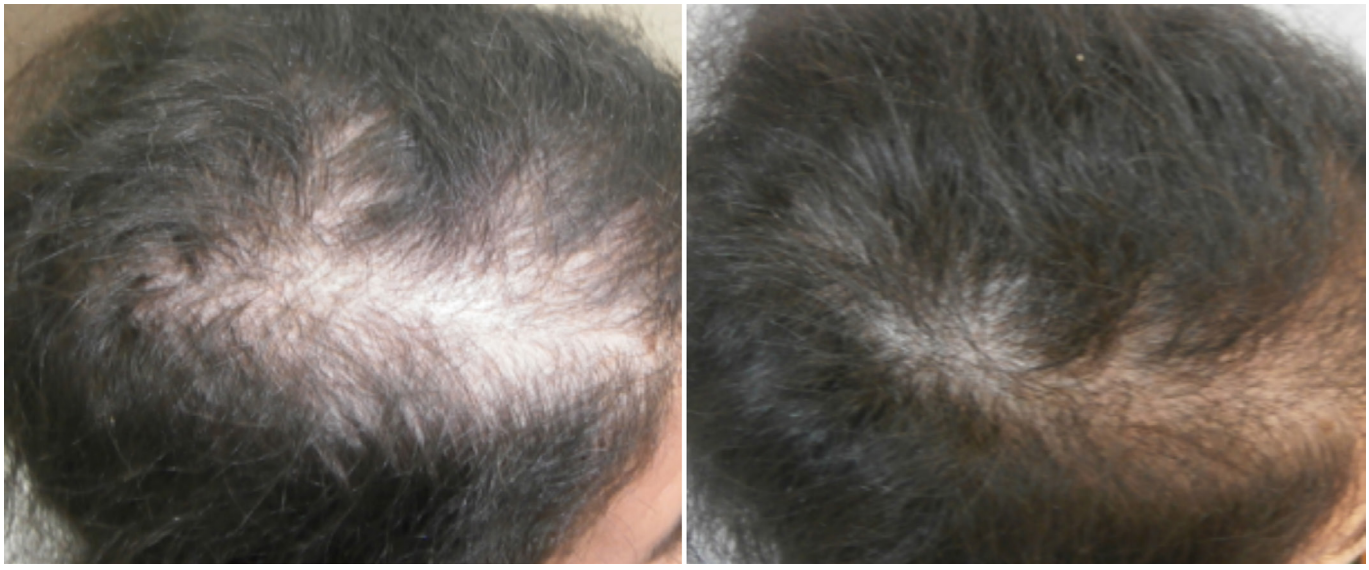

Figure 3. *Before (left) and after (right).*

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Answer to Prof. Sito's comment on the article

"Height enhancement using hyaluronic acid and minimally invasive technique"

by Martusciello D. published on the Journal Aesthetic Medicine Volume 3 · Number 2 · April - June 2017; pages 57-64.

Dr. Sito's observations have been very useful and I will clarify the points he queried as follows:

About item one, first of all we have to emphasize that our findings must be considered on the basis of a virtual cavity that we have found when hyaluronic acid had been injected therefore at a later time and after it had been inflated.
It would however be very interesting to validate this or try to find Martusciello pouch at the dissection table as well.

About item two, it had already been considered to produce further work in which we would specify point by point all the results of the increment in height achieved with the mini-invasive technique we have described; of course we will take this observation into consideration and use a centimeter grid.

With regard to item three, we have defined the procedure described as mini-invasive but since the beginning we have well specified that the patient could have experienced pain (despite pain being a subjective perception).
This point was so important to us that we have included a table reporting the degree of pain of the procedure *(Table 2)*.
It is however important to mention that if a patient does not strictly follow the prescribed drug therapy pain can be severe.
This is one of the reasons why we have started with a certain schedule of drug administration but we have since improved our experience in this regard so that in the next publication we will better specify the prescription schedule, what we suggest to use, drugs and dosing regimen for a duration of just three days and how to make the procedure easily tolerable.

Item four: as often reported in the article, the Ilizarow technique and the one described are not comparable because the first is a severely painful surgical technique although it allows for large and stable increments in height while our technique is mini-invasive, the procedure is easy and fast and allows to correct modest etherometry (about 2cm) and is suggested for people who wish to obtain moderate height increments. It is suitable for aesthetic purposes. As mentioned earlier, our procedure is less stable and, as hyaluronic acid is absorbing material, the duration is limited.

Patient Nº	PAIN
1	3
2	4
3	3
4	4
5	5
6	5
7	4
8	3
9	4
10	3
11	4
12	3
13	3
14	3
15	5
16	4
17	4
18	4
19	4
20	3
21	4

Table 2. *Pain referred evaluation. Range is between 0, no pain, and 5, very painful.*

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Courses and Congresses

2017

12-14 May - Rome (Italy)
38th National Congress of the Italian Society of Aesthetic Medicine
12th National Congress of the Italian Academy of Aesthetic Medicine
Venue: Congress Centre Rome Cavalieri
President: E. Bartoletti
E-mail: sime@lamedicinaestetica.it - congresso@lamedicinaestetica.it
Web: www.lamedicinaestetica.it

19 August - Montevideo (Uruguay)
16th Congress of the Uruguayan Society of Aesthetic Medicine
Regency Way Montevideo Hotel
President: A. Elbaum
E-mail: info@sume.com.uy - medicinaesteticacongreso@gmail.com
Web: www.sume.com.uy

8-9 September - Paris (France)
38th National Congress of Aesthetic Medicine and Dermatologic Surgery
French Society of Aesthetic Medicine
French Association of Morpho-Aesthetic and Anti-Aging Medicine
National Institute of education in aging prevention
President: J.J. Legrand
Web: www.sfme.info

22-24 September - Almaty (Kazakhstan)
9th National Congress of Aesthetic Medicine and Plastic Surgery
Kazakhstan Association of Aesthetic Medicine and Plastic Surgery
President: G. Zhumatova
E-mail: info@estetic.kz
Web: www.estetic.kz

6-8 October - Warsaw (Poland)
17th International Congress of Aesthetic and Anti-Aging Medicine
Polish Society of Aesthetic Medicine and Anti-Aging
Hilton Warsaw Hotel and Convention Center
President: A. Ignaciuk
E-mail: sekretariat@ptmeiaa.pl
Web: www.icaam.pl

27-29 October - Istanbul (Turkey)
21th World Congress of Aesthetic Medicine
Turkish Society of Aesthetic Medicine
Rumeli Caddesi Durak Apt N° 2, D.7 - Nisantasi, Istanbul - Turkey
President: H. Subasi
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Web: www.estetiktipdernegi.org.tr

3-4 November - Lausanne (Switzerland)
15th Congrès De La Société Suisse De Médecine Esthétique
5th Congrès De La Société Suisse De Chirurgie Esthétique
Le Beau-Rivage Palace à Lausanne
President: S. Le Huu
Venue: Le Beau-Rivage Palace à Lausanne
Web: www.ssme.ch/congres-ssme-et-ssce-2017

10-11 November - Santiago (Chile)
11th Chilean Congress of Aesthetic Medicine
Chilean Association of Aesthetic Medicine
Santiago - Chile
Hotel Intercontinental - Las Condes
President: G. Marzullo
E-mail: contacto@creativaproducciones.cl
Web: sochme.cl/congresomedicinaestetica

10-12 November - Miami (Florida – USA)
14th Annual AAAMC
American Academy of Aesthetic Medicine Congress
Aesthetic Medicine from Research to Practise
JW Marriott Miami
President: M. Delune
E-mail: delegate@aaamed.org
Web: www.aaamed.org/congress2017

24-25 November - Toronto (Canada)
CAAM 14th Annual Conference
American Academy of Aesthetic Medicine Congress
Hilton Toronto
President: R. Van Aardt
E-mail: info@caam.ca
Web: www.caam.ca

30 November - 1th December – Algeri (Algeria)
16th National Congress of Aesthetic Medicine and Surgery
Algerian Society of Aesthetic Medicine
Hotel Mercure Alger
President: M. Oughanem
E-mail: Oughanem_m@hotmail.com
Web: www.same-dz.com

7-9 December - Estoril, Lisbon (Portugal)
2nd National Congress of Aesthetic Medicine
Aesthetic and Anti-Aging Medicine Society of Portugal
Hotel Palacio Do Estoril
President: J.P. Vale
E-mail: congressonacional@spme.pt
Web: www.spme.pt

2018

22-24 February - Malaga (Spain)
33th National Congress SEME
Spanish Society of Aesthetic Medicine
Palacio de Ferias y Congresos
President: P. Vega
E-mail: seme2018@pacifico-meetings.com
Web: www.seme2018.org

2-3 March - Mexico City (Mexico)
15th Mexican Scientific Congresso of
Aesthetic Medicine and Antiaging
15th Venezuelan Congress of Aesthetic Medicine
Mexican Scientific Society of Aesthetic Medicine
Aesthetic Medicine Society of Venezuela
Presidents: J-B. Miller Kobisher and V. García Guevara
Venue: Pepsi Center - World Trade Center, Mexico City
E-mail: congresoacademico@ippc.mx

4-6 April - Buenos Aires (Argentina)
12th Pan-American Congress of Aesthetic Medicine
28th Argentinian Congress of Aesthetic Medicine
Argentinian Society of Aesthetic Medicine - SOARME
President: R. Pinto
Venue: Auditorio de la Universidad Católica Argentina
Av. Alicia Moreau de Justo 1680
Puerto Madero - Buenos Aires
Web: www.soarme.com

19-21 April - Brussels (Belgium)
12th European congress UIME
National congress SBME-BVEG
Belgian Society of Aesthetic Medicine and Lasers
Radisson Blu Royal Hotel
President: J. Hebrant, H. Cartier
E-mail: info@aesthetic-medicine.be
Web: sbmebveg.be

10-12 May - Pretoria (South Africa)
12th Aesthetic Medicine Congress South Africa
AMCSA 2018
Aesthetic and Anti-Aging Medicine Society of South Africa
CSIR Convention Centre, Pretoria
President: J. Van Niekerk
E-mail: info@aesthmed.co.za
Web: www.aesthmed.co.za

18-20 May - Rome (Italy)
39th National Congress of the Italian Society of Aesthetic Medicine
13th National Congress of the Italian Academy of Aesthetic Medicine
Venue: Congress Centre Rome Cavalieri
President: E. Bartoletti
E-mail: sime@lamedicinaestetica.it - congresso@lamedicinaestetica.it
Web: www.lamedicinaestetica.it

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